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STUDY OF THE MECHANISM OF BONE DISEASE IN HYPOPHOSPHATEMIC GLYCOSURIC OSTEOMALACIA*

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MALACIC bone disease in association with renal glycosuria, aminoaciduria, hyperphosphaturia and increased excretion of organic acids is a rare disorder known as the Fanconi or DeToni-Fanconi syndrome. Although this apparently inborn error of metabolism has been most frequently identified with resistant rickets, several recent articles have associated the syndrome with adult osteomalacia. The bizarre combination of metabolic disorders associated with renal tubular defects is of general interest, for study of these abnormalities contributes to basic understanding of amino-acid metabolism, acid-base balance, tubular reabsorptive mechanisms and bone and mineral metabolism. This investigation is concerned mainly with the last, for lack of identification of the cause of the skeletal demineralization has impeded the application of universally effective therapeutic measures. Various facets of the Fanconi syndrome have been developed in several comprehensive papers, notable among these being the reports of McCune, Mason and Clark (1), Stowers and Dent

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(2), Anderson, Miller and Kenny (3), Milne, Stanbury and Thomson (4), and Linder, Bull and Grayce (5).

The patient discussed in this report was a 42-year-old Army sergeant whose entire medical history prior to this investigation has been described as an example of "Milkman's" syndrome (6). During his previous study multiple pseudofractures were demonstrated but the cause of the bone disease could not be identified. Lack of response to large doses of vitamin D, the presence of glycosuria with normal glucose tolerance and the fact that a renal tubular defect had not been considered, indicated the desirability of further metabolic study.

METHODS

The patient was hospitalized on the Metabolic Service of Walter Reed Army Hospital. Laboratory studies were conducted at the Army Medical Service Graduate School and at Georgetown University School of Medicine. Urines were collected for periods of twenty-four hours, treated with suitable preservatives and stored under refrigeration until analysis. Stools were collected for periods of four to six days, using carmine and charcoal alternately as markers.

Chemical analyses were conducted by standard procedures. Calcium in blood, urine and stools was measured by the respective methods of Clark and Collip (7), Van Slyke and Sendroy (8), and McCrudden (9); phosphorus by the method of Fiske and Subba-Row (10) and by uranium acetate titration (11). Urinary titratable acidity was measured by the method of Henderson (12), urinary ammonia by that of Folin and Bell (13), and urinary organic acids by that of Van Slyke and Palmer (14). Blood amino nitrogen was measured by the method of Folin as modified by Frame, Russell and Welhelmi (15); for urinary amino nitrogen the methods of both Sahyun (16) and Albanese (17) were employed. Sodium and potassium were measured by the flame photometer, chloride by the method of Schales and Schales (18) and CO_2 by that of Van Slyke (19). The technique of Kunkle (20) was used for the determination of blood magnesium. The Beckman pH meter was used for the determination of blood and urine pH and for a portion of the titratable acid determinations.

In the study of mineral excretion two rotating diets were given, neutral in ash and containing constant amounts of nitrogen, calcium and phosphorus as calculated from standard food tables.

RESULTS

The more frequent cause of osteomalacia, steatorrheal disease, had been sought for during previous hospitalizations. Stool fat was not excessive in amount, there was no evidence of deficiency of fat-soluble vitamins A and K, glucose absorption was normal and x-ray studies showed no evidence of either pancreatic or intestinal disease. The administration of 200,000 to 300,000 units of vitamin D_2 daily for from two to three months resulted in no chemical or radiologic improvement, ruling out vitamin-D deficiency and indicating the absence of vitamin-D resistance. Thus it seemed probable that the osteomalacia was associated with a primary renal defect.

TABLE 1. IMPORTANT LABORATORY CHANGES

Bone and mineral			
Calcium	10.0 mg./100 cc.		
Phosphorus	1.8 mg./100 cc.		
Alk. P-tase	16.0 B.U./100 cc.		
X-ray exam.	Pseudofractures; "osteoporosis"		
Urine			
Glycosuria	0–16 Gm./day	[0]	
Hyperphosphaturia	28 cc./min./1.73 Sq. M.	[7–14]	
Organic aciduria	1.8 mEq./Kg./day	[0.5–1]	
Amino aciduria	400–900 mg./day		
pH	6.1		
Ammonia	40 micro Eq./min.		
Titratable acid	21 micro Eq./min.		
Acetone	positive		
Volume	3000 cc./day		
Blood			
Cations (mEq./L)		*Anions (mEq./L)*	
Sodium	141.0	Chloride	102.0
Potassium	5.0	CO^2	28.0
Calcium	5.0	Phosphate	1.0
Magnesium	5.0	Protein	17.0
		Sulfate	1.0 (estim'd)
	156.0		149.0
		Estimated organic acid = 7.0 mEq./L	

The more striking laboratory findings, which were in general characteristic of the Fanconi syndrome, are shown in Table 1.

Bone roentgenograms showed numerous band-like areas of decreased density, commonly referred to as pseudofractures and considered typical of osteomalacia, as well as generalized radiotranslucence of bone. The concentration of minerals in the blood was consistent with the diagnosis of osteomalacia.

Glucose, identified by the osazone test, was found in the urine in varying amounts, at times being absent and at most amounting to 18 Gm./day. During the initial portion of this study urinary glucose excretion averaged 15 Gm./day but later it decreased in amount and at times was completely absent. Although urinary amino acids were not markedly excessive in amount, there was a moderate increase, averaging from 400 to 900 mg. a day, except when the urine was free of glucose. Single dimensional paper chromatography conducted in the manner described by Dent (21) revealed

a moderate increase in amino acids when tested urines contained glucose, but an essentially normal pattern of amino acids was observed when the urine was aglycosuric. In this case there did not appear to be the strict quantitative correlation between glycosuria and aminoaciduria that Dent has observed, but it is obvious that lack of intense aminoaciduria would make such a correlation difficult to establish. Analyses by two-dimensional chromatograms later in the study, at which time the urine contained only small amounts of glucose, showed no serine or other hydroxy amino acids. Tyrosine was present in several of the analyzed urines. In addition to amino acids other organic acids were present in the urine in excessive amounts, lactic and pyruvic acid being especially prominent.

Urinary phosphorus excretion exceeded 1,000 mg./day while the patient had a phosphorus intake of 1,000 to 1,800 mg./day. As shown in Figure 1,

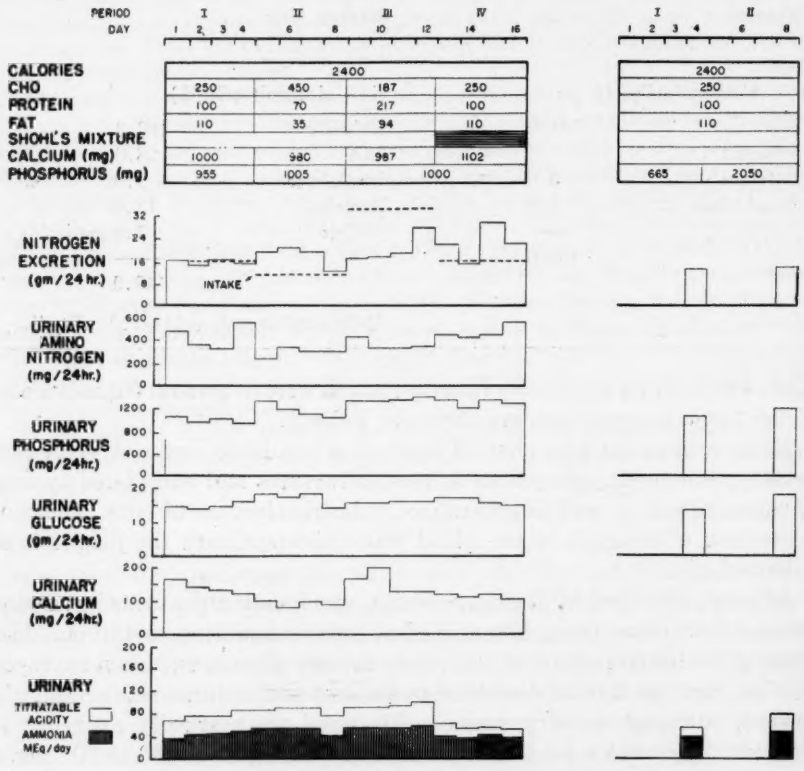

FIG. 1. Changes in urinary excretion during variation of an isocaloric diet, administration of alkali and change in intake of phosphate.

a high level of excretion of phosphorus was maintained when phosphorus intake was reduced.

The blood pH, CO_2, chloride, sodium and potassium were normal and the urinary excretion of ammonia and the urinary titratable acidity were within normal limits, consistent with the urinary pH and the excretory load of phosphate and organic acids. Alkali therapy brought about slightly decreased excretion of ammonia and a substantial reduction in urinary titratable acidity. Positive results with tests for acetone were frequently noted. In view of the minimal urinary wastage of glucose, it is apparent that ketonuria could not be attributed to the ketosis known to accompany the caloric deficit in severe renal glycosuria. Polyuria and hyposthenuria were constant, urine volume averaging about 3,000 cc./day.

Because treatment of the osteomalacia in the group of related disorders known as the DeToni-Fanconi syndrome has been generally unsatisfactory, more precise identification of the cause of the bone disease was deemed essential. Studies were based on evaluation of four possible mechanisms: 1) loss of metabolites necessary for normal nutritional balance, 2) excessive primary urinary loss of calcium, 3) a disorder of organic acid metabolism, or 4) excessive phosphate excretion.

The term "nutrition" is used here in the broad sense of indicating either protein deficiency, which might produce a deficient bone matrix, or loss of specific amino acids necessary for normal mineral metabolism. As shown in both Figure 1 and Figure 3, the patient was in nitrogen balance and responded normally to variation in nitrogen intake. Variation in the ingested amounts of the major components of an isocaloric diet resulted in no major change in urinary excretion of glucose, amino acid or phosphate (Fig. 1). Calcium excretion, which was not markedly excessive, showed a tendency to decrease during a low protein intake and increase during a high one. No change in excretion of glucose, amino nitrogen or phosphorus resulted from administration of alkali in the form of Shohl's mixture[1] (22), 75 cc. per day; however, a decrease in titratable acidity did occur and, to a lesser degree, in ammonia.

Dent has suggested that preferential loss of hydroxy amino acids, especially serine which is necessary for phosphate transport, may be a significant cause of the bone disease in glycosuric rickets (21). The lack of positive evidence to support this suggestion, the absence of excessive serine excretion in this patient, as well as the fact that serine can be synthesized, render this theory unlikely.

[1] Shohl's mixture:
Citric acid 140.0
Sodium citrate 98.0
Water q. s. ad 1000.0

The second primary cause of the bone disease would be excessive loss of calcium. Hypercalcinuria might be expected if the renal disorder in the Fanconi syndrome included the defect seen in the syndrome of renal hyperchloremic acidosis (23). However, the presence of an acid urine and the absence of hyperchloremia and nephrocalcinosis rendered this association very unlikely. It has been suggested that patients with glycosuric rickets may lose fixed body base, including calcium, because of overloading of the renal base-sparing mechanisms by the excessive organic acids present in the urine (23). Because of the tremendous capacity for renal ammonia production, such would appear unlikely. However, although it now appears probable that the defective ammonia production in the syndrome of nephrocalcinosis with tubular acidosis is the result of lack of acid stimulation rather than of a specific defect of ammonia formation (24), it was considered possible that the latter might be an associated defect in the Fanconi syndrome. This possibility appears to be ruled out, as shown in Figure 2, by the fact that this patient's ammonia production increased readily in response to the stimulus of an acid load. Certainly this increase

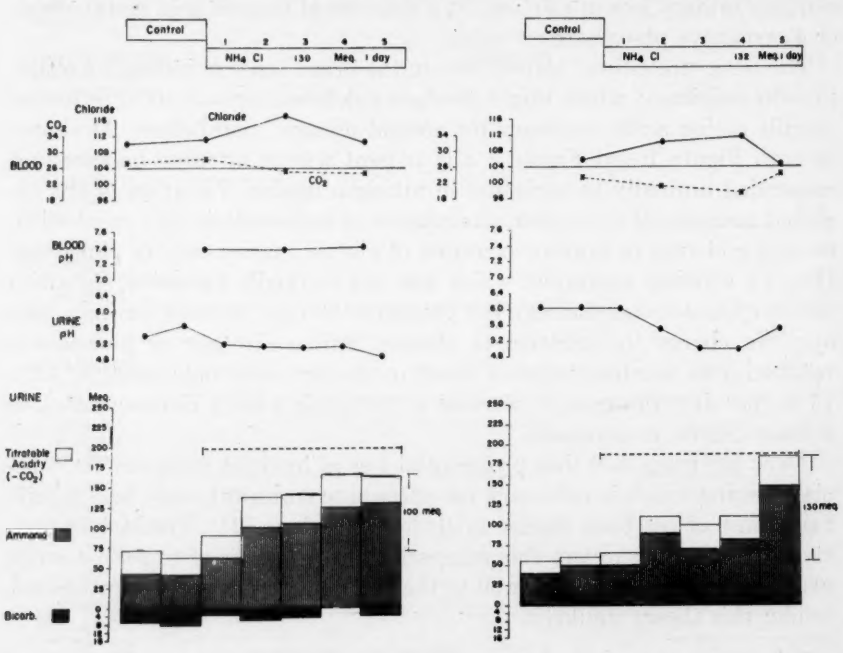

FIG. 2. The response to an acid load in the patient of this study (A) compared with that of a control patient (B).

in base-sparing capacity was more than sufficient to compensate, not only for a greatly excessive organic acid load, but also for a diet which was strongly acid-ash in composition. Ammonia production in the Fanconi syndrome has been frequently reported as excessive in amount but it does not appear that such production is necessarily an abnormality, considering the large amounts of endogenous acid presented to the distal tubule for excretion. Moreover, reported studies wherein ammonia excretion is related to total nitrogen excretion in the urine are obviously fallacious, because of the fact that ammonia production bears no direct relationship to excretion of other nitrogenous substances. The same may be said for the relation of amino-acid nitrogen to nitrogen excretion, for (as shown in Figure 1) urinary excretion of ammonia and amino nitrogen bear no relationship to that of total nitrogen. Milne and his co-workers have advanced the idea that the increased urinary ammonia is due to the large amounts of amino acids present in the renal tubule which may be used to form ammonia (4). In this case there was insufficient aminoaciduria to allow evaluation of such a hypothesis and the patient did not show systemic acidosis. In short, ammonia production was normal for the urinary pH and increased markedly following acid stimulation. Thus no mechanism attributable to acidosis or impaired ammonia synthesis could be advanced to suggest primary hypercalcinuria as a cause of the osteomalacia.

The third possibility deserving consideration as a cause of demineralization of bone, is an abnormality of organic acid metabolism. Evidence that the Fanconi syndrome is more than a renal disorder is afforded by its close relationship with cystinosis (21) and apparent connection with some instances of hepato-lenticular degeneration (25), as well as the finding of an excess of undetermined anion in the blood of certain patients with this syndrome. In addition it should be noted that no instance of the DeToni-Fanconi syndrome has shown nephrocalcinosis. Absence of renal calculi in a disorder which manifests excessive urinary calcium and phosphorus excretion, frequently in the presence of a weakly acid urine, suggests that the excessive urinary organic acids may prevent precipitation of calcium salts by forming a poorly ionized complex with calcium. Such protective action has been suggested in association with an increase in urinary citrate (26). It is not inconceivable that a high blood level of organic acids, especially citrate, might have a similar action in preventing the normal deposition of calcium in bone. However, in the case under discussion, no excess of blood organic acid was demonstrable and quantitative comparison of blood cations and anions revealed no discrepancy attributable to an excess of undetermined anion.

The fourth disorder worthy of consideration as a primary cause of osteomalacia, is that of excessive urinary excretion of phosphorus. Marked

phosphaturia has been observed in the reported cases of the Fanconi syndrome, with the exception of that studied by Stowers and Dent (2), and their patient was believed to have attained phosphate equilibrium after marked skeletal mineral loss. McCune, Mason and Clark (1) and Stowers and Dent consider the phosphaturia as the most probable cause of osteomalacia. Milne and his associates believe that hyperphosphaturia is a common finding in all types of malacia, irrespective of etiology, and that it decreases after effective therapy of the bone disease. Their tabulated data, however, are not convincing with respect to a significant change in phosphate clearance after treatment. These observers also noted that the elevated phosphate clearance in one case of glycosuric rickets fell to normal after successful therapy with vitamin D, but Anderson was unable to confirm that finding in his patient (27). Our patient showed a phosphate clearance of 28 cc. per minute (normal in this laboratory, 7 to 14 cc./min.), as well as continued high phosphate excretion following drastic reduction

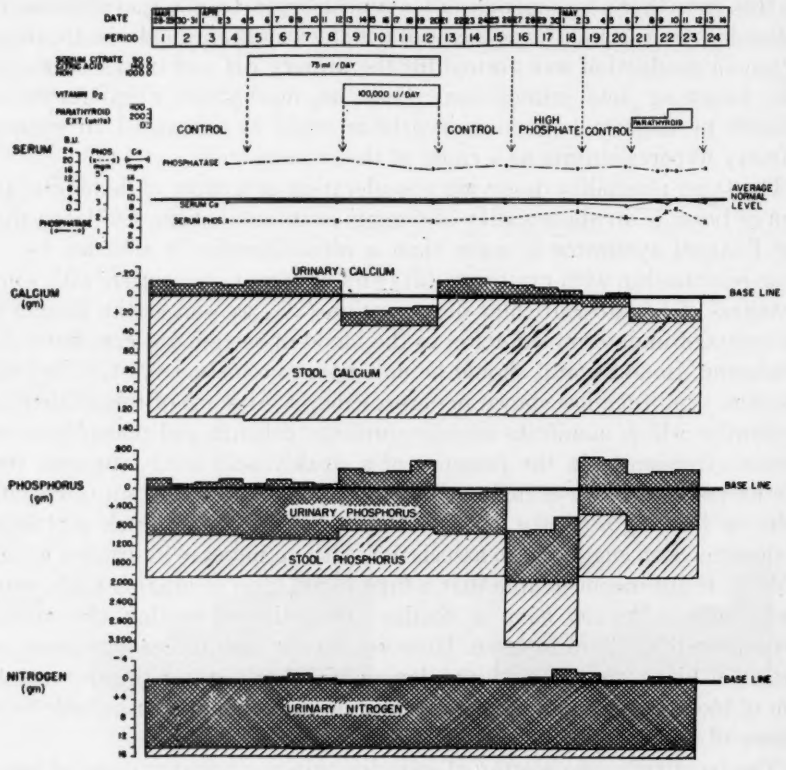

FIGURE 3

of phosphate intake (Fig. 1). To investigate this matter further a more complete metabolic study was conducted. The results of this study are shown in Figure 3, charted by the method of Bassett as popularized by Albright.

The patient was given a diet of constant calcium and phosphorus content, neutral in ash and containing a constant amount of protein. Two daily diets were alternated throughout the study. After a preliminary period of stabilization, urine and stool collections were started. The former were collected for periods of twenty-four hours, and stools were collected in periods of from four to six days. The results of this study may be summarized as follows:

During a control period of eight days the patient was in negative calcium and phosphorus balance; throughout the study he was in normal nitrogen balance. The blood mineral values were characteristic of osteomalacia. During the next eight days alkali therapy in the form of Shohl's mixture[1] was added (22). There was no change in calcium or phosphorus balance or in the blood mineral values. During the next eight days the patient received 100,000 units of vitamin D each day, in addition to the sodium citrate-citric acid preparation. This resulted in positive calcium balance but phosphorus excretion increased. After a control period of six days, phosphorus intake was increased by the addition of sodium acid phosphate to the diet. This resulted in a very positive phosphate balance, and a calcium balance which nearly became positive. During this period of time the serum phosphorus level rose from 1.9 to 2.5 mg. per 100 ml.

Prior to the conduct of this balance study an Ellsworth-Howard test had been performed (28). Intravenous administration of parathyroid extract resulted in a substantial increase in urinary phosphorus excretion and a rise in serum phosphorus concentration of from 1.8 to 2.6 mg. two hours after the injection. Consequently it appeared desirable to test the effect of parathyroid hormone further. The period of high phosphate administration was followed by another short control period, following which parathyroid hormone was administered intramuscularly, 200 units per day for three days and 400 units for two days. During this time there occurred a decrease in fecal calcium excretion and, although phosphorus excretion was of the same magnitude observed during vitamin-D therapy, the serum phosphorus level rose sharply. Tweedy has supplied some evidence that parathyroid hormone may exert an influence on fecal calcium excretion (29), but elevation of serum phosphorus concentration in the face of increased urinary excretion of that substance would suggest that the less popular theory of parathyroid hormone action, *i.e.*, directly on bone, was operative in this individual.

Thus it appears from these observations that excessive phosphate excre-

tion is the dominant mineral defect in the DeToni-Fanconi syndrome. However, identification of the mechanism of the phosphaturia is vital in order that the assumption may safely be made that the excessive excretion is not purely a secondary manifestation of demineralization of bone. Consideration was therefore given to four possible causes of increased phosphorus excretion: 1) acidosis, 2) competitive interference with reabsorption, 3) hyperparathyroidism secondary to hypocalcemia, and 4) a specific disturbance of renal tubular reabsorption.

Although systemic acidosis, known to induce increased phosphate excretion, has been present in several instances of glycosuric rickets, acidosis was not present in this case. Furthermore the administration of alkali resulted in no appreciable change in phosphate excretion.

The second possibility considered was competitive interference with renal phosphate reabsorption by other substances, such as glucose, amino acids or organic acids. This appeared very unlikely in view of the fact that the amount of urinary phosphate was not quantitatively related to urinary levels of either glucose or amino acid, nor was it affected by changes in the basic components of an isocaloric diet.

The third possibility, secondary hyperparathyroidism, is difficult to rule out, for in other types of osteomalacia there exists excellent pathologic evidence for the existence of overactive parathyroid glands (30). However, utilization of the concept of hyperparathyroidism in explanation of phosphaturia necessitates demonstration of a primary cause that would lower the serum calcium level, proof of which could not be provided by these studies. Moreover, the response of this patient to the Ellsworth-Howard test provided indirect evidence against the presence of overactive parathyroid glands. The intravenous administration of 2 cc. of parathyroid extract resulted in an increase of phosphate clearance from 27 to 47 cc./min. Urinary excretion of phosphorus (mg. per hour) increased in a fashion similar to that of normal persons reported in the literature and studied in this clinic. If the renal tubule were under excessive stimulation by endogenous parathyroid hormone, one would not expect a marked increase in phosphate excretion following the administration of a small dose of exogenous hormone.

Consequently the fourth possible cause of hyperphosphaturia, namely, a specific primary defect in tubular reabsorption of that mineral, appears more probable. The authors can offer no other good explanation and the limited histologic evidence available in the literature indicates the presence of a proximal tubular lesion in other cases of glycosuric rickets (2). In addition, all the foregoing positive evidence indicates a specific limitation of tubular phosphate reabsorption.

DISCUSSION

Several of the well studied cases of glycosuric rickets have shown metabolic abnormalities which might be significant in the causation of bone disease. However, these abnormalities, with the exception of hyperphosphaturia, vary markedly in degree. In some cases excessive aminoaciduria, with especially striking excretion of serine and threonine, has been prominent; in others, including the case reported here, urinary excretion of amino acids has been much less pronounced. Some cases of the Fanconi syndrome show severe acidosis, with features suggestive of the syndrome of hyperchloremic acidosis, but others do not. Increased excretion of organic acids has been emphasized in some cases, but not in others. Consequently, the fact that our patient demonstrated such marked bone disease in the absence of acidosis and marked aminoaciduria, lends more credence to the data indicating the primacy of phosphate loss in producing demineralization of bone. With no evidence for a primary loss of calcium, it therefore appears probable that urinary calcium excretion may be increased because of the loss of normal bone structure, calcium becoming superfluous in the face of inadequate amounts of phosphorus available for bone formation.

It is difficult to reconcile the apparent benefit resulting from alkali therapy in the case studied by Anderson and the rapid improvement in Milne's case after alkali and vitamin-D treatment, with the studies in our patient, for neither vitamin D nor Shohl's mixture appeared to benefit mineral balance. Although Milne's patient was extremely acidotic, the case studied by Anderson had an essentially normal electrolyte pattern.

Dent has suggested that in all the basic renal tubular disorders which manifest malacia (except possibly hyperchloremic nephrocalcinosis) phosphate loss is of primary importance; the other absorptive defects vary quantitatively and qualitatively among the different tubular disorders (31). This view would appear to be the one which is best correlated with the data we have presented. It is obvious, however, that the group of renal tubular disorders, which include the Fanconi syndrome, may show any number of absorptive defects, and that findings in any one case are not strictly applicable to all others.

The theoretical causes of osteomalacia are decreased calcium absorption, increased calcium loss, decreased phosphorus absorption and increased phosphate loss. The existence of the first three possibilities has been supported by clinical and experimental observations. Acceptance of the concept that increased phosphate loss is the dominant defect in the Fanconi syndrome provides a clinical example of the fourth.

SUMMARY

1. Evidence is presented to suggest that the demineralization of bones which occurs in association with the DeToni-Fanconi syndrome is secondary to excessive urinary excretion of phosphorus.

2. It appears probable that the phosphate loss is the result of defective reabsorption of this substance on the basis of a specific renal tubular abnormality.

3. Lack of response to vitamin-D therapy in patients with the Fanconi syndrome may be attributed to the fact that although this vitamin causes increased calcium retention it also increases the urinary excretion of phosphate.

4. Acceptance of the probability that decreased tubular reabsorption of phosphorus is the cause of bone disease in the Fanconi syndrome allows provision of examples of each of the theoretical causes of malacic bone disease.

Acknowledgment

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HYPERCALCEMIA SECONDARY TO BONE METASTASES FROM CARCINOMA OF THE BREAST

I. RELATIONSHIP BETWEEN SERUM CALCIUM AND ALKALINE PHOSPHATASE VALUES

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HYPERCALCEMIA is not uncommon in patients with skeletal metastases from breast cancer. This complication occurs spontaneously in about 10 per cent of these patients and in about 20 per cent as a result of hormonal therapy (1, 2, 3, 4). The causal relationship between hormonal therapy and the production of hypercalcemia was established by prior investigation, in which it was found that the alternate administration and withdrawal of the sex hormones could induce successive incidents of hypercalcemia in the same patient (1). On occasion, an incident of spontaneously occurring or regressing hypercalcemia may coincide with the administration of the sex hormones.

The following sequence in the development of hypercalcemia resulting from bone metastases has been postulated and recently subjected to metabolic investigation (5) by Laszlo and co-workers: 1) excessive breakdown of bone, with liberation of calcium and phosphate ions into the blood stream, due to the rapidly expanding osteolytic neoplastic process; 2) hypercalcinuria and hyperphosphaturia; 3) hypercalcemia, when the renal capacity for excreting calcium is exceeded by the filtered load; and 4) renal impairment.

In contrast to osteolytic lesions which release calcium and phosphate ions (Fig. 1—Reaction A), osteoblastic metastases take up these ions and the process may be associated with decreased urinary excretion of calcium and phosphate ions and increased serum alkaline phosphatase levels (Fig. 1—Reaction B).

It is generally accepted that alkaline phosphatase is produced at the site of new bone formation (6, 7) and that, in the absence of hepatic or biliary tract disease (8), the blood serum values of this enzyme reflect at-

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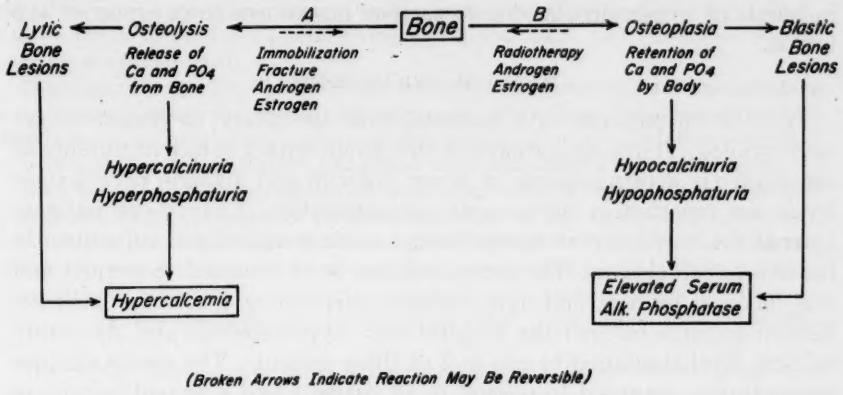

FIG. 1. Schematic representation of metabolic activity in osteolytic and osteoblastic metastases.

tempts at bone regeneration. Histochemical studies of osteoblastic bone neoplasms have shown greatly increased alkaline phosphatase activity in these tissues, occurring concomitantly with elevated serum concentrations of the enzyme, whereas the study of osteolytic tumors has revealed normal or decreased alkaline phosphatase values in the lesions and in the blood serum (9). Although hepatic metastases may cause an increase in the level of serum alkaline phosphatase (6, 10), an unpublished study indicates that such an increase tends to be steadily progressive, whereas there are sharp fluctuations of the serum concentration of this enzyme in the presence of osseous metastases (11).

Patients with metastatic osseous disease may exhibit a fluctuating course of remission and exacerbation of these lesions. Changes in the metabolic activity, or in the rate of progression or regression of metastases, may occur spontaneously; they may also occur as a result of various therapeutic procedures such as roentgenotherapy, the deprivation of sex hormones (castration), or the administration of sex hormones (estrogens and androgens). There may be alternate periods of great osteolytic activity with hypercalcemia and hypercalcinuria, followed by intervals of bone repair with calcium retention and deposition in areas of osteolysis (12). Usually osteoplasia is associated with elevated serum alkaline phosphatase values, and osteolysis with normal or lowered values. In a previous investigation there was suggestive evidence of an inverse relationship between the level of serum calcium and serum alkaline phosphatase activity in patients with osseous metastases from carcinoma of the breast (13).

The present communication presents a study of the relationship between the serum concentrations of alkaline phosphatase and of calcium in

incidents of hypercalcemia due to osseous metastases from cancer of the breast.

MATERIAL AND METHODS

Forty-seven patients with hypercalcemia secondary to breast cancer were studied. Thirty-six patients of this group with a sufficient number of simultaneous determinations of serum calcium and alkaline phosphatase levels are reported in the present communication. Twenty-one patients entered the hospital with normal serum calcium values and subsequently became hypercalcemic. The serum calcium level returned to normal and was followed by a second hypercalcemic incident in 5 of these patients. Fifteen patients entered the hospital with hypercalcemia and the serum calcium level continued to rise in 2 of these patients. The serum calcium concentration returned to normal in 13 patients and a second episode of elevated levels occurred in 5 of these patients.

Serum calcium concentrations were determined by the Clark-Collip modification of the Kramer-Tisdall method, which gives normal values of from 9 to 11 milligrams per

TABLE 1. ANALYSIS OF DEVELOPING AND SUBSIDING EPISODES OF HYPERCALCEMIA

Amt. of decr. or incr. in serum alkaline phosphatase activity	Type of episode			Type of metastasis		
	Spontaneous	Estrogen-induced	Testosterone-induced	Episodes in patients with blastic metastases	Episodes in patients with lytic metastases	Total
Group 1—Hypercalcemia, developing (33 episodes)						
Definite fall (over 2 B.U.)	6B*	2B	2B	10		
	3L†		7L		10	
						20 (61%)
Slight fall (0.5–2 B.U.)	1B			1		
	4L	3L	2L		9	
						10 (30%)
Inconsistent	2B		1B	3		
						3 (9%)
Total	*16*	*5*	*12*	*14*	*19*	*33*
Group 2—Hypercalcemia, subsiding (32 episodes)						
Definite rise (over 2 B.U.)	6B	1B	3B	10		
	3L		9L		12	
						22 (69%)
Slight rise (0.5–2 B.U.)	2B			2		
	5L		1L		6	
						8 (25%)
Inconsistent			2L		2	
						2 (6%)
Total	*16*	*1*	*15*	*12*	*20*	*32*

* B—Blastic metastases.
† L—Lytic metastases.

100 ml. Serum alkaline phosphatase activity was determined by the Bodansky method, 2 to 4 units (B.U.) per 100 ml. representing the normal range, and values over 5 units being considered elevated.

Hypercalcemia was considered to be present when the serum calcium concentration exceeded 12 milligrams per 100 ml. in two or more determinations. Coinciding with variations in serum calcium values, a change in the serum alkaline phosphatase activity of 2 or more units was termed *definite*. Changes of lesser magnitude were termed *slight*.

Each fluctuation of the serum calcium level which could be correlated with a change in the serum alkaline phosphatase value was considered an individual episode.[1] Sixty-five such episodes were encountered in these 36 patients. For convenience in evaluation the 65 episodes were divided into two groups. *Group 1* comprised the 33 episodes in which hypercalcemia appeared or, if present, increased during the period of observation. *Group 2* comprised the 32 episodes in which high serum calcium levels returned to normal.

The various direct inciting causes of the hypercalcemic incidents, as well as the type of bone reaction noted in roentgenograms taken at the time of an episode, are given in Table 1.

RESULTS

Group 1. Increasing serum calcium values associated with decreasing alkaline phosphatase activity (33 episodes)

Hypercalcemia was associated with decreased serum alkaline phosphatase activity in 30 (91 per cent) of the 33 episodes in this group. *Definite* decreases in serum alkaline phosphatase values were found in 20 of these episodes (61 per cent) and *slight* decreases were encountered in 10 episodes (30 per cent). An increase of from 2 to 3 Bodansky units was noted in 3 epidoses (9 per cent) (Table 1).

Of probable significance is the observation that 11 of the hypercalcemic incidents studied in Group 1 were preceded by a decrease in the serum alkaline phosphatase activity of at least 1.5 Bodansky units before any significant increase in serum calcium concentration was noted (Fig. 2). In the remaining cases of this group sufficient determinations were not made prior to recognition of hypercalcemia to establish the possible presence of a premonitory fall in the serum alkaline phosphatase activity.

Group 2. Decreasing serum calcium values associated with increasing alkaline phosphatase activity (32 episodes)

A fall in serum calcium levels toward normal limits was associated with a rise in serum alkaline phosphatase values in 30 (94 per cent) of the 32 episodes in this group. *Definite* increases in serum alkaline phosphatase activity were encountered in 22 episodes (69 per cent), *slight* increases in 8 episodes (25 per cent) and no consistent change in 2 episodes (6 per cent). There was a premonitory rise in the serum alkaline phosphatase activity preceding the fall of the serum calcium level in only 3 patients.

[1] An episode refers either to a *rise* from normal to hypercalcemic levels, or to a *drop* from hypercalcemic to normal levels.

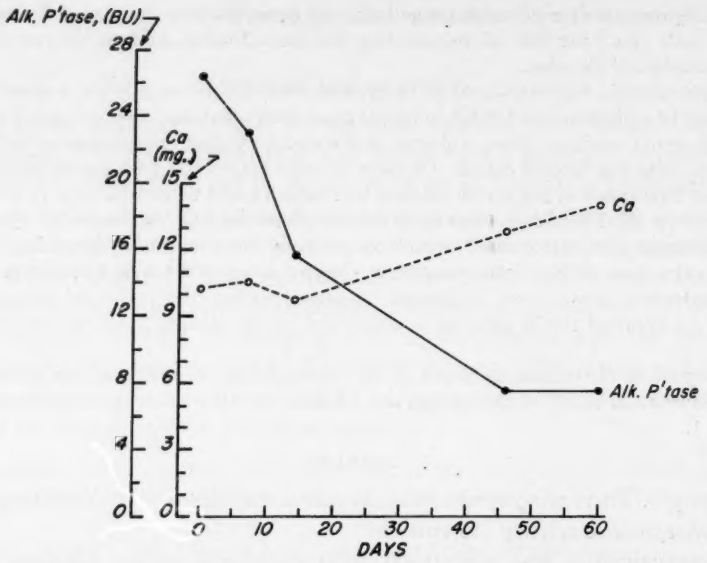

FIG. 2. Representative case, demonstrating the gradual fall in serum alkaline phosphatase activity before the serum calcium level began to rise.

A representation of the inverse relationship between serum alkaline phosphatase and calcium values in patients of both groups is shown in Figure 3. The bar graphs (Figs. 4 to 7) show the data from representative cases and illustrate the inverse relationship between serum alkaline phosphatase and calcium concentrations during the onset and regression of hypercalcemia, whether spontaneous, testosterone-induced or estrogen-induced. An example is included of hypercalcemia induced and treated by testosterone propionate.

COMMENT

Wide fluctuations in metabolic activity, varying from periods of rapid bone breakdown through interludes of relative metabolic quiescence to periods of active bone repair, are found in patients with osseous metastases from breast carcinoma. Although these metastases are usually osteolytic, predominantly osteoblastic metastases may occur in at least 8 per cent of cases (14). Many patients present concurrent lytic and blastic areas in different parts of the skeletal system and microscopic studies reveal that lesions, which appear to be entirely osteolytic on roentgenographic examination, may contain areas of bone regrowth and repair (5, 6, 10).

The roentgenogram shows a composite of destructive and reparative

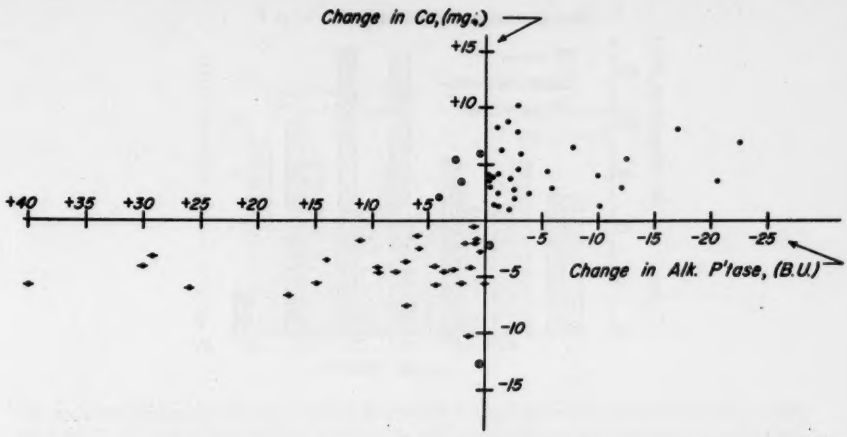

FIG. 3. Demonstration of the inverse relationship between serum calcium and alkaline phosphatase levels in hypercalcemia secondary to bone metastases.

In this figure there is plotted the difference (in mg. per 100 ml.) between the highest and lowest calcium values measured for each episode of calcium fluctuation against the difference (in Bodanksy units) between the highest and lowest serum alkaline phosphatase values for the same episode.

Group 1 (developing hypercalcemia) episodes lie in the upper right quadrant. A rise in calcium is associated with a drop in alkaline phosphatase activity. Group 2 (subsiding hypercalcemia) episodes lie in the lower left quadrant. A fall in calcium is accompanied by a rise in serum alkaline phosphatase activity.

The episodes in Group 1 with inconsistent alkaline phosphatase changes are indicated by an X within a circle. These patients had predominantly osteoblastic metastases on roentgenographic study. The episodes in Group 2 with inconsistent serum alkaline phosphatase changes are indicated by concentric circles. These episodes occurred in patients with roentgenographic evidence of purely osteolytic metastases.

events but is limited with regard to information about the metabolic equilibrium of the patient. In this series an appreciable number of patients with roentgenographic evidence of osteoblastic changes were in a metabolic phase of marked osteolysis, demonstrated by the presence of hypercalcemia and decreasing serum alkaline phosphatase values (Table 1).

It is possible that a more definite index of the metabolic status of the patient at any given time may be furnished by serial determinations of serum calcium and alkaline phosphatase levels in conjunction with roentgenography. A consistent decrease in the serum alkaline phosphatase activity may indicate impending hypercalcemia and require the immediate application of remedial measures.[2] Since hypercalcemia is recognized as a grave complication of metastatic bone disease with a serious morbidity

[2] Remedial measures have been described in a previous publication (1).

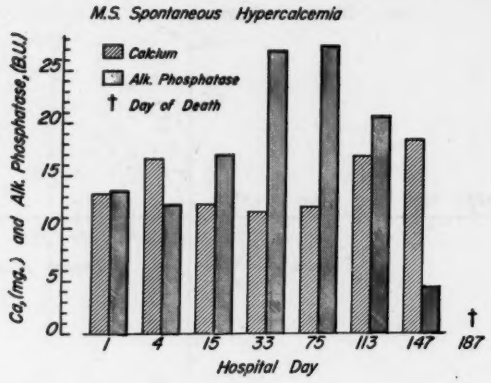

FIG. 4. This and the following figures illustrate the inverse relationship between the serum calcium and alkaline phosphatase values.

M.S., a 38-year-old postmenopausal woman had undergone a radical mastectomy one and one-half years previously. At the time of admission to the Montefiore Hospital she had extensive osteolytic metastases, and soon after admission an incident of spontaneous hypercalcemia occurred.

leading frequently to death, a premonitory fall in the serum alkaline phosphatase concentration, which may precede hypercalcemia, can be of practical interest and importance. More data, however, are required to establish the degree of reliability of this diagnostic sign.

The serum calcium and alkaline phosphatase values varied inversely in 60 (92 per cent) of the 65 episodes studied, but the relationship was not proportional. The degree of change in the alkaline phosphatase level was

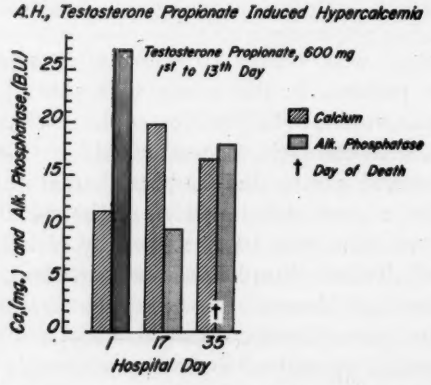

FIG. 5. *A.H.*, a 49-year-old postmenopausal woman with widespread osteolytic metastases exhibited hypercalcemia following the administration of testosterone propionate.

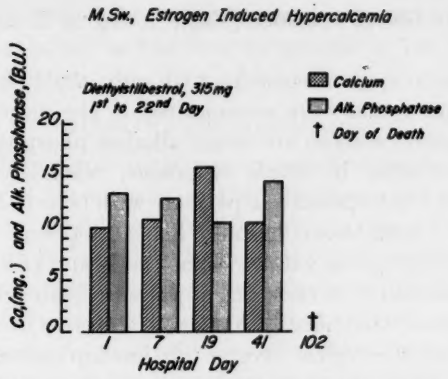

FIG. 6. *M. Sw.*, a 45-year-old postmenopausal woman with extensive osteolytic and osteoblastic disease exhibited hypercalcemia following the administration of estrogen.

often greater or smaller than the degree of change in the serum calcium level (Fig. 3). Patients with roentgenographic evidence of osteoblastic metastases frequently showed initially high serum alkaline phosphatase activity and a more marked capacity to alter this activity in association with changes in the serum calcium level than did patients with osteolytic lesions.

Definite falls in the serum alkaline phosphatase activity at the onset of hypercalcemia were encountered in 10 of the 14 episodes (Table 1, Group 1) and *definite* rises associated with falling calcium values, were en-

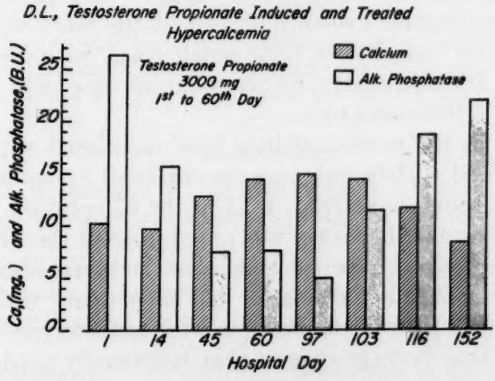

FIG. 7. *D.L.*, a 32-year-old woman with extensive osteolytic metastases received no benefit from castration. The administration of testosterone propionate induced hypercalcemia. An unsuccessful attempt was made to correct the hypercalcemia by the continued administration of the androgen. Upon termination of testosterone therapy the serum calcium returned to normal levels.

countered in 10 of the 12 episodes (Table 1, Group 2) in this class of patients.

The greater percentage of episodes with only *slight* changes in alkaline phosphatase serum values were encountered in the patients with lytic lesions. They frequently showed low serum alkaline phosphatase activity and little capacity to alter it. *Slight* decreases, when hypercalcemia was present, were noted in 9 episodes in patients with lytic lesions and in only 1 episode associated with blastic lesions (Table 1—Group 1). *Slight* rises in serum alkaline phosphatase values, when the serum calcium levels fell to normal, were observed in 6 episodes in patients with lytic lesions and in 2 episodes associated with blastic changes (Table 1—Group 2).

Episodes without the typical inverse relationship between serum calcium and alkaline phosphatase concentrations were encountered in patients in both groups. Three episodes, in which an increase in serum alkaline phosphatase values of 2–4 Bodansky units was noted with the development of hypercalcemia, occurred in 2 patients (Group 1) with roentgenographic evidence of extensive osteoblastic activity. On each of these occasions a return of the serum calcium to normal concentrations was accompanied by a further marked increase in the serum alkaline phosphatase values (10 to 40 Bodansky units). It would seem, therefore, that the small rise in the serum alkaline phosphatase activity with the onset of hypercalcemia in patients with great osteoblastic potentiality is a manifestation of decreased bony proliferation under these circumstances.

Two episodes without the inverse relationship between the serum calcium and alkaline phosphatase values occurred in 2 patients (Group 2) with no roentgenographic evidence of osteoblastic reaction. They demonstrated little or no capacity for bone repair since, with decreasing serum calcium values, the serum alkaline phosphatase range was constantly between 3.5 and 4.5 Bodansky units.

Fluctuations in the serum calcium level associated with testosterone administration and withdrawal were accompanied by a *definite* change in the alkaline phosphatase activity in 21 of the 27 episodes (Groups 1 and 2—Table 1), whereas this change was noted in 18 of the 32 episodes associated with spontaneous hypercalcemia. This occurred despite the greater incidence of osteoblastic metastases in the patients with spontaneous hypercalcemia (17) than in the patients with testosterone-induced hypercalcemia (6). These findings suggest that hormonally produced hypercalcemia is associated more frequently with a marked change in the serum alkaline phosphatase value than is spontaneous hypercalcemia.

The extensive fluctuations of serum alkaline phosphatase activity with inverse relationship to the serum calcium levels in the present series, occurring repeatedly in many patients, strongly suggest that liver metas-

tases had relatively little effect on the changes observed. Further evidence is furnished by the failure to find liver metastases in 4 of these patients on postmortem examination.

SUMMARY AND CONCLUSIONS

1. A study of 36 patients with hypercalcemia secondary to bone metastases from cancer of the breast disclosed 65 episodes in which a change in serum calcium level could be correlated with a change in alkaline phosphatase activity. In 60 episodes (92 per cent) the relationship was inverse.

2. These results suggest that roentgenograms supplemented with serial determinations of the concentration of serum calcium and alkaline phosphatase may furnish a more adequate evaluation of the metabolic status of osseous metastases at any one time than roentgenography alone.

3. There was suggestive evidence that the onset of hypercalcemia is preceded by a premonitory fall in the serum alkaline phosphatase activity. If this relationship can be substantiated it would constitute a valuable diagnostic method for the recognition of impending hypercalcemia.

4. Patients with some osteoblastic metastases exhibited a more pronounced fluctuation of serum alkaline phosphatase activity in association with alterations in the serum calcium level than did patients with exclusively osteolytic lesions.

5. There was suggestive evidence that testosterone-induced hypercalcemia was associated with wider fluctuations of serum alkaline phosphatase activity than was spontaneous hypercalcemia.

Acknowledgments

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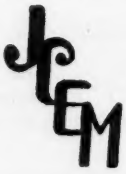

EXPERIENCE WITH LONG-TERM CORTISONE THERAPY IN CONGENITAL ADRENOCORTICAL HYPERPLASIA

REPORT OF 4 CASES*

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CONGENITAL adrenocortical hyperplasia is a condition marked by a high degree of androgenicity. It is manifested as pseudohermaphrodism in the female and macrogenitosomia praecox in the male. Characteristically, there is an increased urinary excretion of 17-ketosteroids. Treatment prior to 1950 was unsatisfactory; in fact, the intensity of the virilization was often such that many clinicians considered it preferable to transform some of the more severely masculinized genetic females into passable males than to condemn them to live as unhappy and inadequate females. The male prototype was usually better off, unless he had the misfortune to be the victim of an associated electrolyte disturbance, which at times superimposes the addisonian complex upon the adrenogenital syndrome. Usually his only complaint, once maturity was attained, was his short, even at times dwarfed, stature due to premature epiphyseal closure, the result of early excessive androgenicity.

Early in 1950, Sprague *et al.* (1) reported that the administration of cortisone in man reduced the urinary excretion of 17-ketosteroids. It followed inevitably that attempts would be made to utilize therapeutically this androgenic depressant activity of cortisone.

In August 1950, there appeared in the Endocrine Clinic of Mt. Zion Hospital, 2 siblings, a 13-year-old female pseudohermaphrodite and her 6-year-old brother with macrogenitosomia praecox. Here was an opportunity to test whether suppression of the adrenal might halt, or at least retard, the boy's untimely sexual development and too rapid skeletal growth and maturation. What benefit, if any, might accrue to the 13-year-old girl, whose epiphyses were already fused and whose main complaint was intense hypertrichosis, was not clear at the time. However, Wilkins (2), who had already pioneered the experimental work on this problem, stated in a

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personal communication that administration of cortisone to female pseudohermaphrodites depressed the androgenic activity of the hyperplastic adrenal sufficiently to permit the occurrence of normal female maturation, otherwise held in abeyance. Accordingly, cortisone was administered to both children and to 2 adult female pseudohermaphrodites as well.

These 4 cases are not being reported merely to add confirmation to the fact that cortisone is effective in reducing the androgenicity in congenital adrenocortical hyperplasia. This fact has been well documented by the work of others (2–6). Rather, it is the intent of the author to review the clinical experiences, *both good and bad*, encountered in the long-term treatment of this disease with cortisone, in the hope of aiding physicians contemplating its use, as well as to provide additional evidence for later, final evaluation of the true efficacy of this form of treatment.

REPORT OF CASES

Case 1

J.W. (M.Z.H.), aged 13 years, was born in Germany of a mother who had two miscarriages prior to this full-term pregnancy. The infant weighed 5½ pounds at birth and seemed to develop normally at first. Although clitoral enlargement was noted at 18 months, the child was 5 years old before medical consultation was sought. Noninterference was advised, and the suggestion was made that the child be raised according to the sex it seemed to be. At 11 years of age, while living in the Russian zone of occupation, the patient was hospitalized for study. Here, frequent clinical demonstrations of her abnormalities caused much emotional and mental trauma. In 1949, in Munich, an exploratory laparotomy to establish the sex of the child was performed. The operation revealed an infantile uterus and large pale ovaries, resembling those described by Stein and Leventhal (7). The adrenals were said to be normal to abdominal palpation. A vaginal plastic operation was performed, but the hypertrophied clitoris was not disturbed. Based on the results of microscopic examination of ovarian tissue removed at operation, a diagnosis of fetal ovaries was made. However, Dr. Arthur Hertig, who later examined the histologic specimens, described enlargement of the ovary, thickening of the cortex, increased stroma, a paucity of primordial ova, and an abnormally active theca interna (Fig. 1e).

The past history of the child was not otherwise unusual. She had never shown any symptoms suggesting the addisonian complex. She had mild measles, mumps and chicken pox between the ages of 3 and 4 years, eczema between the ages of 2 and 4, and a tonsillectomy at the age of 4. Furuncles had appeared frequently during the previous year.

There was no family history of consanguinity or intersexuality, nor any instance of endocrine disease, except in the 6-year-old male sibling.

J.W. was sulky, stubborn, moody and antisocial. She resisted medical help and wished only to be left alone. She complained of excessive fatigue, required much sleep, was nervous and irritable, and especially quarrelsome at home.

Physically, she was long-waisted, narrow-hipped and flat-chested (Fig. 1a). Her height was 61½ inches, weight 115 pounds, and blood pressure 120/70 mm. Hg. The span was 63½ inches; the lower body measurement from pubis to floor was 31 inches. Coarse hair was present on cheeks, chin, neck and upper lip, as well as over the body generally

FIG. *1. Case 1.* Female pseudohermaphrodite, aged 13 years. *a.* Note short stature, short legs, broad shoulders, narrow hips, and absence of mammary development. *b.* Note masculine facies, hirsutism and prominent larynx. *c.* Note enlarged clitoris, absence of labia minora, and marked hirsutism. *d.* Diodrast hysterosalpingogram demonstrating uterus and patency of tubes. *e.* Ovarian biopsy. Note thickened cortex and increased stroma. *f.* Breast development after seven months of oral cortisone therapy.

(Fig. 1b). There was a male pubic escutcheon and heavy axillary hair. Mild acne was present on the face. The larynx was prominent and well developed. Her voice was low-pitched, husky, and unpleasant in quality; it cracked and broke frequently.

The hypertrophied clitoris measured 4 cm. in length and 6 cm. in circumference (Fig. 1c). Although the labia majora were well developed and pigmented, labia minora were absent; in their place was a parchment-like membrane, in the center of which was a reddened stellate opening, too small to admit the tip of a little finger. The urethral orifice was in the normal female position. Examination under anesthesia revealed a vagina approximately 8 cm. in length, so tight that it barely admitted a small endoscope. Diodrast injected into the vaginal canal successfully outlined cervix, uterus and tubes, demonstrating patency throughout (Fig. 1d). The ovaries were palpable bilaterally. Vaginal smears showed an absence of estrogenic effect.

Laboratory data. Results of blood count and urinalysis were normal. Circulating eosinophils numbered 209 per cu. mm. A glucose tolerance test, with 100 Gm. of glucose given orally, gave the following results: fasting blood sugar, 80 mg. per 100 ml.; ½ hour, 112 mg.; 1 hour, 73 mg.; 2 hours, 85 mg.; and 3 hours, 65 mg. per 100 ml. A 24-hour urinary gonadotropin determination[1] was questionably positive at 5 m.u. The urinary 17-ketosteroid excretion[2] was 88, 61, and 76 mg. per twenty-four hours, respectively, on three different occasions. Dehydroisoandrosterone was absent in the one specimen tested.[3] Assays of 17-hydroxycorticosteroids[4] in the blood on two occasions were reported as 3.5 gamma and 2.7 gamma per 100 ml. (normal, 4–5 gamma per 100 ml.). Bone-age studies when the patient was 13 showed all epiphyses united. No abnormalities were demonstrated by roentgenograms of the skull or intravenous urograms.

Discussion. Although the diagnosis of female pseudohermaphrodism might have been suspected on the basis of the genital anomalies, early virilization and the existence of a male sibling with macrogenitosomia praecox, the exploratory laparotomy removed all possible doubt. It seemed fairly certain that the primary aberration lay in the adrenal cortex and that cortisone therapy should be tried.

Management was beset with difficulties, due to previous psychologic trauma; however, a satisfactory rapport was finally achieved and the patient agreed to cooperate in a trial of cortisone therapy. This was begun on April 3, 1951. Cortisone was given orally in divided daily doses. The amount and duration of therapy are shown in Chart I. As may be seen, the 24-hour urinary 17-ketosteroid output dropped immediately from the pretreatment average of 75 mg. to a level of 6 mg. Within a month breast development could be noted, and the hair growth seemed less vigorous. From May 15 to August 10, the dosage was inadequate as gauged by 17-ketosteroid output, and the breasts stopped growing. With the resumption of more adequate dosage on September 5, the urinary 17-ketosteroid excretion was again depressed, but not to the low levels reached initially. However, the breasts resumed development (Fig. 1f) and menarche occurred. The clitoris seemed smaller.

The patient was well maintained on a regimen of 50–75 mg. of cortisone a day for ten months (September 1951 to July 1952). The urinary 17-ketosteroid excretion remained at high normal levels (average, 14 mg.). Further depression was desirable, however, before a favorable effect on the hirsutism could be anticipated. Since Wilkins (12) had

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[1] Method of Klinefelter, Albright, and Griswold (8).

[2] Method of Drekter, Pearson, Bartczak and McGavack (9).

[3] Method of Allen, Hayward and Pinto (10).

[4] Method of Nelson and Samuels (11).

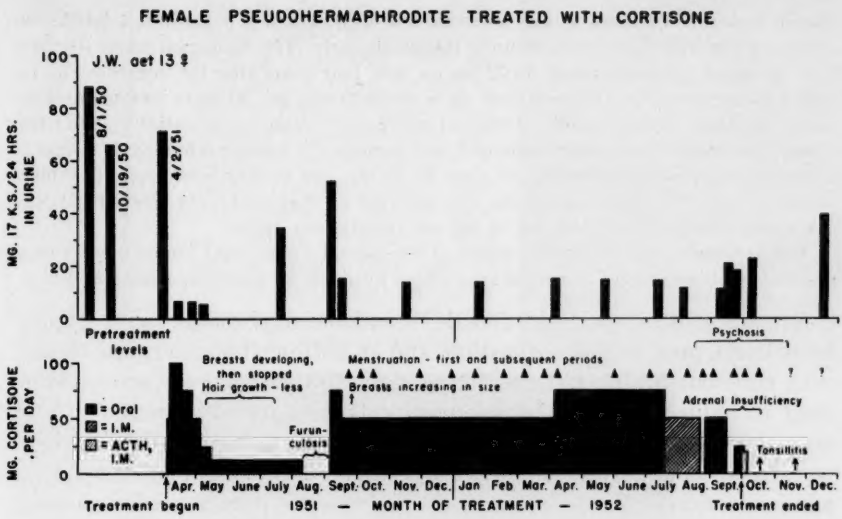


CHART I

shown that patients required smaller doses of cortisone intramuscularly than they did orally, to achieve the same result, the route of administration was changed on July 18 but the dosage was maintained at 50 mg. daily. Two weeks later the urinary 17-ketosteroid output was 11.5 mg. per twenty-four hours. At this time the patient began to complain of dizziness, palpitation and fear of crowds, and to withdraw within herself. She stopped attending school and spent her days lying around. (In retrospect, one wonders if these symptoms might have been due to potassium loss; however, since the patient failed to appear in the clinic, no tests were run.) On August 22, the patient discontinued the parenteral injections of cortisone on her own initiative; four days later she resumed the oral preparation. Palpitation and dizziness persisted. The blood pressure was 110/90, and the pulse 92 per minute. Menstrual periods, which had occurred at fairly regular intervals, increased in frequency (August 8–13, August 22–25, and again on September 2).

By September 16, it was found necessary to discontinue cortisone because of the schizoid psychosis. Almost immediately the patient experienced anorexia, nausea and increased weakness and dizziness. The tonsils, which were said to have been removed in childhood, became extremely hypertrophic and inflamed. The girl's unusual pallor at this time suggested the possibility of silent bleeding from the gastrointestinal tract. Blood counts and stool examination failed to confirm this suspicion. The symptoms of which she complained and the dramatic tonsillar enlargement were assumed to be evidence of a relative adrenocortical insufficiency, secondary to prolonged cortico-depressant therapy. Desoxycorticosterone acetate linguets®, 2 mg. buccally, and cortisone, 12.5 mg. orally twice daily, were prescribed. The patient was hospitalized on September 23; she was depressed, withdrawn and uncooperative. On September 24, the serum sodium concentration was elevated to 148.5 mEq./L and the serum potassium depressed to 3.5 mEq./L. Desoxycorticosterone acetate and cortisone were discontinued, and potassium triplex, 5 cc. twice daily, and a low-sodium diet were instituted. By October 1,

the concentration of serum sodium measured 140 mEq./L and of potassium, 4.4 mEq./L. Corticotropin was then given, 25 units intramuscularly. The eosinophil count dropped from an initial 110 per cu. mm., to 72 per cu. mm. four hours after the injection—an insufficient response. For the next three days corticotropin gel, 20 units intramuscularly, was given daily. The symptoms of adrenal insufficiency disappeared entirely within two weeks. The tonsils remained hypertrophic and periodically became inflamed. By October 7, the 17-ketosteroid excretion had risen to 25 mg. per twenty-four hours. Complete recovery from the schizoid psychosis had occurred by November. On December 7, the urinary 17-ketosteroid output was 40 mg. per twenty-four hours.

Unfortunately, the subsequent status of the patient's menstrual function and breast development is unknown, since she is no longer available for follow-up studies.[5]

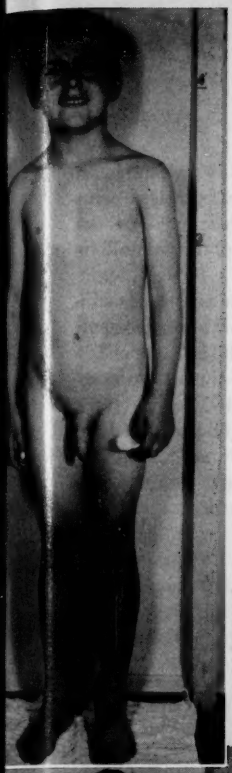
Summary. A 13-year-old female pseudohermaphrodite, with typical body build, pronounced virilization, and an antisocial manner, was treated with cortisone orally. Fifty to 75 mg. daily in divided doses proved sufficient to reduce the high, initial urinary 17-ketosteroid excretion to high normal levels. The breasts developed, and menarche occurred. The vigorous growth of abnormal hair was only slightly attenuated, however. After administration of 50 mg. of cortisone daily by the intramuscular route for two weeks, a psychosis developed. Following discontinuance of cortisone, transient symptoms of adrenocortical insufficiency appeared, including a remarkable hypertrophy of the tonsils. This patient received, over a period of seventeen months, a total of 23,500 mg. of cortisone orally and 1,700 mg. intramuscularly. This amount and duration of therapy had no permanent effect on the endogenous production of corticotropin nor on the responsiveness of the adrenals, for within two and a half months after cessation of therapy, the urinary 17-ketosteriod excretion had risen to abnormally high, but not quite to pretreatment levels. This is in line with the experience of Wilkins (13).

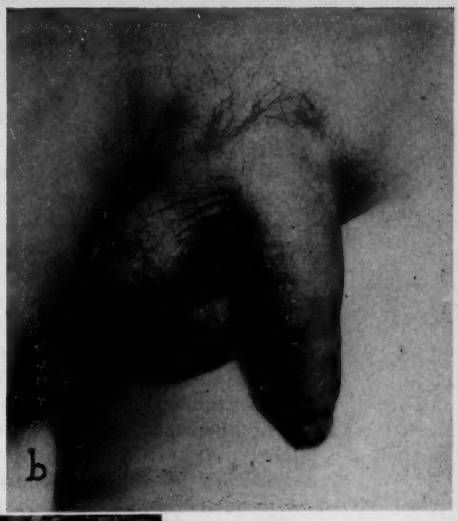
Case 2

A.W. (M.Z.H.) was 6 years and 3½ months old when first seen in September 1950. Born at full term, he weighed 7.7 pounds and was considered normal, except that his genitalia were unusually well developed and purplish. Although he had always been large for his age, the greatest increment in growth had occurred during the preceding year (5–6 years of age). Despite his conspicuous somatic and sexual precocity, apparently the boy had never been considered abnormal. Attention was focused upon him only after his 13-year-old sister was recognized to be a female pseudohermaphrodite. The sole suggestion of concomitant addisonian complication in the child was that he consumed large amounts of salt.

The boy was 52¾ inches tall (average normal height for a 10-year-old) and weighed 67½ pounds. His facial and skeletal appearance corresponded to that of a boy of about 11 years; his genital development was perhaps slightly more advanced (Fig. 2a). His psy-

[5] A report from the mother in June 1953, stated that the breasts had ceased enlarging but that menstrual periods continued.





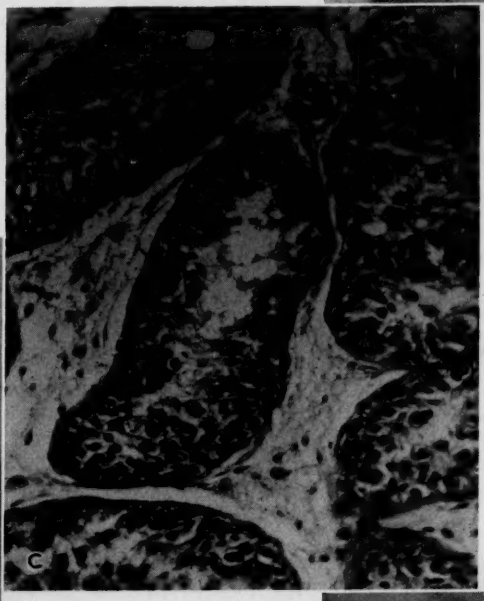


FIG. 2. *Case 2.* Macrogenitosomia praecox in boy, aged 6 years. *a.* Chronologic age, 6 years, 4 months; height, $52\frac{3}{4}$ inches; height age, 10 years; bone age, 10–11 years. Note precocious genital development. *b.* Close-up of genitalia. Note enlargement of testes, as well as penis. *c.* Testicular biopsy. Note presence of spermatocytes and occasional interstitial cells. *d.* Age, 7 years, 8 months; after nine months of cortisone therapy. Height, $57\frac{1}{4}$ inches; height age $12\frac{1}{2}$ years; bone age, 12 years. *e.* Genitalia at the age of 7 years, 8 months, after nine months of cortisone therapy.

chologic, mental, and dental ages corresponded to his chronologic age of 6½ years. A mild degree of acne and a small amount of pubic hair were apparent. The uncircumcised penis measured 10 cm. in length and 8 cm. in circumference. Both testes were in a well developed scrotal sac and were the size of very large olives (Fig. 2b). The prostate could be felt rectally as a flat band about 1 by 2 cm.

Laboratory data. A hemogram showed a slight hypochromic anemia and an eosinophilia. The circulating eosinophils numbered 385 per cu. mm.; the urine was normal; the basal metabolic rate was −2 per cent; the blood cholesterol level was 186 mg. per 100 ml. A glucose tolerance test, with 100 Gm. of glucose given orally, gave the following results: fasting blood sugar, 77 mg. per 100 ml.; ½ hour, 122 mg.; 1 hour, 100 mg.; 2 hours, 60 mg.; 3 hours, 54 mg.; and 4 hours, 76 mg. per 100 ml. Urinary gonadotropin values[6]

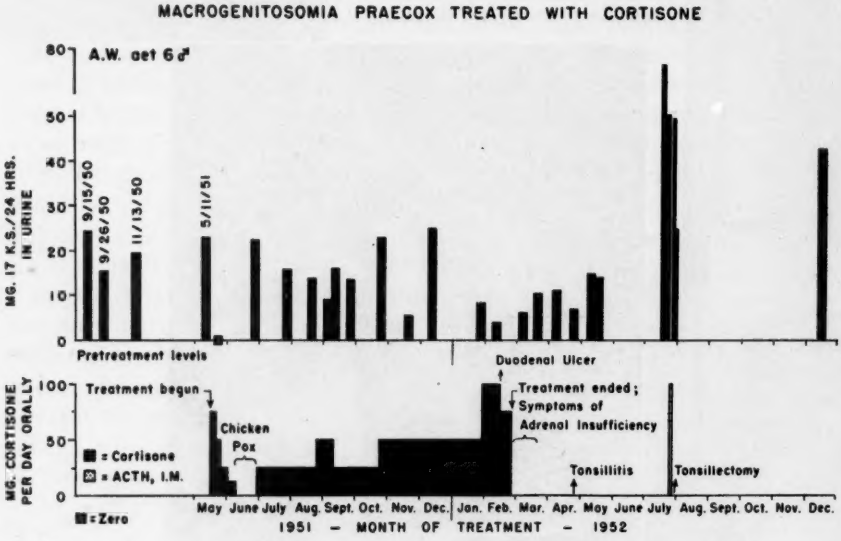


CHART II

were negative at 5 m.u.; the urinary 17-ketosteroids[7] measured 24.3, 15.3, 19.5, and 23 mg. per twenty-four hours on four different occasions (normal for this age, approximately 1.0 mg.). The test for dehydroisoandrosterone[8] gave negative results. Blood assays for 17-hydroxycorticosteroids[9] were 2.8 micrograms and 1.5 micrograms per 100 ml. on two different occasions (normal, 4–5 micrograms per 100 ml.). Roentgenograms of the skeleton showed somewhat irregular maturation; the bone age was estimated as 10–12 years. Intravenous pyelograms showed no evidence of adrenal abnormality and no disease of the urinary tract.

A testicular biopsy made during a circumcision showed numerous seminiferous tubules

[6] Method of Klinefelter, Albright and Griswold (8).
[7] Method of Drekter, Pearson, Bartczak and McGavack (9).
[8] Method of Allen, Hayward and Pinto (10).
[9] Method of Nelson and Samuels (11).

lined with germinal epithelium in which development to the spermatocyte stage could be observed (Fig. 2c). Interstitial cells appeared to be lightly scattered throughout loosely arranged connective tissue.

Treatment. Treatment with cortisone, 25 mg. orally three times a day, was begun on May 15, 1951. After five days the urinary 17-ketosteroid output had dropped to zero. The dosage of cortisone was then gradually reduced at five-day intervals, to 12.5 mg. daily. The correlation of dosage and 17-ketosteroid excretion is shown in Chart II. In this patient, a daily oral dose of 50 mg. of cortisone (25 mg. twice daily) proved inadequate for satisfactory depression of 17-ketosteroid output. When the dose was raised to 75 mg. daily, the boy became hyperexcitable, suffered from insomnia, and his features began to show the typical rounding of Cushing's syndrome. Symptoms of *duodenal ulcer* first appeared in February 1952. The diagnosis was substantiated radiographically a little later. Further cortisone therapy was contraindicated and was abandoned on February 15. One week after withdrawal of cortisone, the boy complained of anorexia, weakness, sore tongue, a large furuncle on the back, and a feeling of his "leg bones being tired." The blood pressure varied between 90/70 and 100/80. The urinary 17-ketosteroid excretion was 6.1 mg. Despite essentially normal serum electrolyte values (sodium, 136 mEq./L and potassium, 4.7 mEq./L), it was assumed that the symptoms were due to temporary adrenocortical insufficiency. Desoxycorticosterone acetate, in the form of linguets®, 2 mg. twice daily, was prescribed. By March 21, he had recovered completely except for residual epigastric pain which cleared somewhat later on a medical regimen for ulcer.

Three months after cessation of cortisone therapy, the urinary 17-ketosteroid output hovered around 14 mg. per twenty-four hours, acne had reappeared, and the testes and prostate seemed larger (Fig. 2d and 2e). In June the tonsils became extremely hypertrophic and inflamed. In July the patient was admitted to the hospital for study of total adrenocortical function preliminary to a contemplated tonsillectomy. Psychologic testing at this time (Stanford Binet Test) showed his mental age to be 8 years and 10 months (chronologic age, 8 years and 1 month). The I.Q. was 109, and in terms of behavior this boy was rated very adaptable.

During the first five days of hospitalization an attempt was made to evaluate the child's tolerance to salt restriction. He was given a salt-poor diet, plus a measured amount of salt, 10 Gm. daily for the first two days, 8 Gm. on the third, and 6 Gm. on the fourth and fifth days. The daily urinary output of sodium chloride was 8.8, 7.8, 5.8, 7.2, and 4.9 Gm., respectively. Salt restriction apparently had no objective or subjective ill effects.

For the next three days corticotropin, 100 units daily, was given by intramuscular injection. No significant changes in electrolyte balance were observed, but the eosinophil and 17-ketosteroid responses were abnormal (Table 1). These results are in harmony with the observations of others and confirmed the clinical impression that the patient did not have the salt-losing form of congenital adrenocortical hyperplasia.

Tonsillectomy was performed on July 29, without incident, and convalescence was uneventful.

Ten months after cortisone was discontinued, the urinary 17-ketosteroids measured 43 mg. per twenty-four hours. At this time the boy was 60 inches tall and weighed 96 pounds. The blood pressure was 90/60. The 12-year molars had erupted. Pubic hair had increased and axillary hair was just becoming apparent; considerable acne covered the back. The penis measured 9 cm. in length and 9.5 cm. in circumference. The right testis was approximately 5 by 3 by 3 cm. and the left 4 by 3 by 3 cm. The bone age was 12–13 years (chronologic age, 8 years and 6 months).

TABLE 1. CASE 2: RESULTS OF ADMINISTRATION OF CORTICOTROPIN
(100 units daily i.m. for 3 days)

	Pre-test period (July 1952)	Test period*	At conclusion of test period
Electrolytes			
Sodium	139 mEq./L 138.5 mEq./L 137 mEq./L		137.5 mEq./L
Potassium	3.5 mEq./L 5.2 mEq./L 4.3 mEq./L		4.2 mEq./L
Chlorides	582 mg./100 ml.		534 mg./100 ml.
Eosinophil count	302 per cu. mm. 148 per cu. mm. 55 per cu. mm. 159 per cu. mm.	110 per cu. mm. 220 per cu. mm. 122 per cu. mm.	
Urinary 17-ketosteroid excretion	77 mg.†/24 hrs. 51 mg./24 hrs.		50 mg./24 hrs. 25.1 mg./24 hrs.

* Consecutive mornings during test period.

† The reason for this unusually high value was not clear, unless it signified a rebound phenomenon. The child was not acutely ill.

Comment. It is claimed (14) that sexual precocity due to congenital adrenocortical hyperplasia may be differentiated from true precocity (constitutional and neurogenic) by the fact that in the former condition the testes usually remain atrophic. Rarely, the testes have shown enlargement, but the enlargement has been ascribed to the presence of adenomatous tissue resembling that of the adrenal cortex. In the case just reported, the size of the testes, and the stage of development noted on microscopic examination, were commensurate with the degree of skeletal maturation.

Treatment extended over a period of nine and half months. A total of 11,825 mg. of cortisone was administered orally. The maximum tolerated daily dose was 50 mg. Hyperexcitability, insomnia and rounding of the facies characteristic of Cushing's syndrome occurred when this amount was exceeded. Despite the fact that the dosage used was not sufficient to depress the 17-ketosteroid output adequately for a great part of the time (Chart II), a satisfactory result in respect to growth and skeletal maturation was achieved. The pretreatment rate of growth for the six-month period (November 1950 to May 1951) was more than twice the normal

rate for boys of this age, as shown by an increment of $2\frac{1}{2}$ inches. During the six-month treatment period (May 1951 to November 1951), the boy grew at one-half the pretreatment rate, or $1\frac{1}{4}$ inches. The bone age was retarded to a relatively greater extent than the growth rate (Chart III). The importance of this observation cannot be overestimated, since according to the height prediction tables of Bayley (15), this boy is now almost certain to achieve normal height.

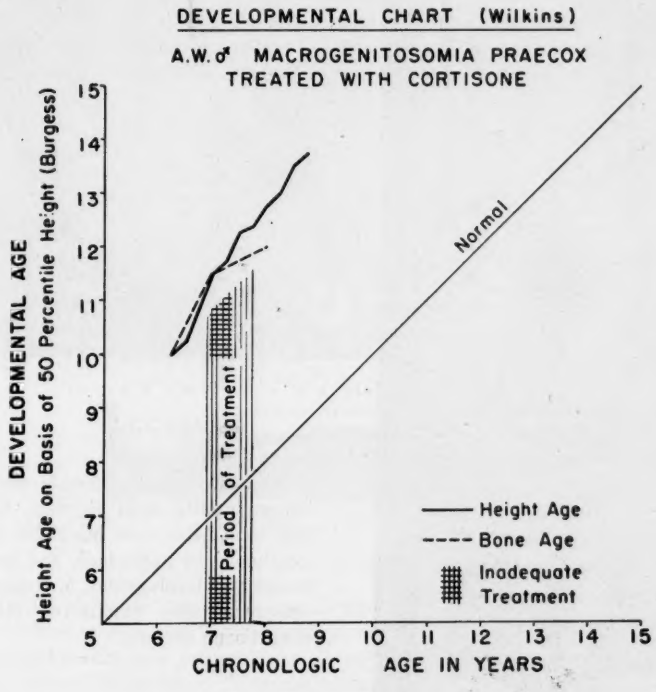


CHART III

Unfortunately, the development of a radiologically proved duodenal ulcer necessitated abandonment of cortisone therapy, since the ulcer undoubtedly was an untoward effect of therapy. Three months after treatment was stopped, growth and sexual maturation resumed their pretreatment pace although the urinary excretion of 17-ketosteriods was greater than it had been initially, proving that nine months of therapy produced only a transient depression of endogenous corticotropic activity and did not impair the subsequent capacity of the adrenal cortex to respond.

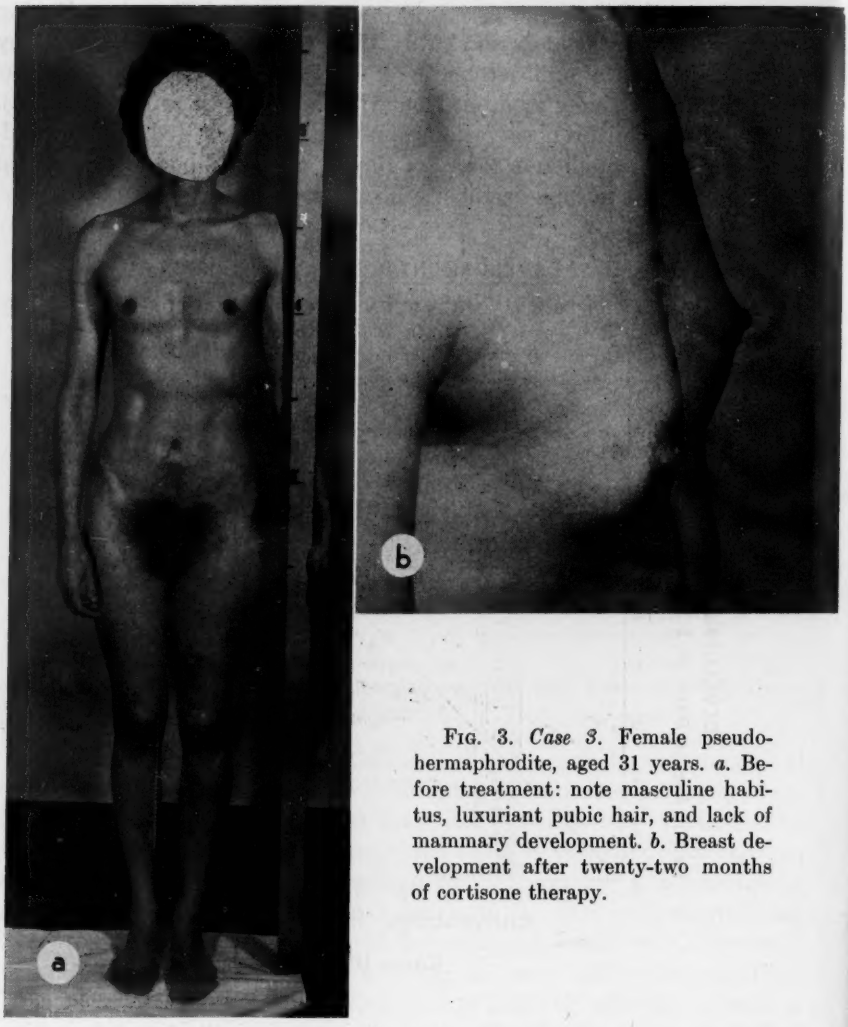


FIG. 3. *Case 3.* Female pseudohermaphrodite, aged 31 years. *a.* Before treatment: note masculine habitus, luxuriant pubic hair, and lack of mammary development. *b.* Breast development after twenty-two months of cortisone therapy.

Case 3

V.P. (private patient) was a 31-year-old single female in whom clitoral hypertrophy was first noted between the ages of 4 and 5 years. Hirsutism developed before the age of 10, and skeletal growth was markedly accelerated. An exploratory laparotomy at the age of 7 revealed normal pelvic organs. One year later the clitoris was amputated. In 1935, when the patient was 15 years old, a partial right adrenalectomy was performed. The postoperative course was particularly stormy. A urinary 17-ketosteroid determination in 1945 was reported as 55 mg. per twenty-four hours. Although no true menstrual

periods had ever occurred, there had been spotting and cramping on the average of once or twice yearly since the age of 15. The family history and past history were otherwise noncontributory.

The patient was a tall, slender, dark-complexioned young woman with a lean masculine build (Fig. 3a). Her voice was low-pitched. She was 66 inches tall and weighed 118 pounds. The blood pressure was 120/80. Scalp, axillary and pubic hair were abundant and coarse; the facial and much of the body hair had been removed successfully by electrolysis. There was a large area of café-au-lait pigmentation over the left dorso-lumbar region. The larynx was large and prominent, the thyroid small. The chest was flat and thin. A minimal amount of breast tissue was palpable; the nipples and areolae were darkly pigmented. A right transverse lumbar scar was evident, and the lower pole of the right kidney was easily palpable. A heavy male pubic escutcheon covered a midline operative scar. The hands and feet were large. The labia majora were well developed

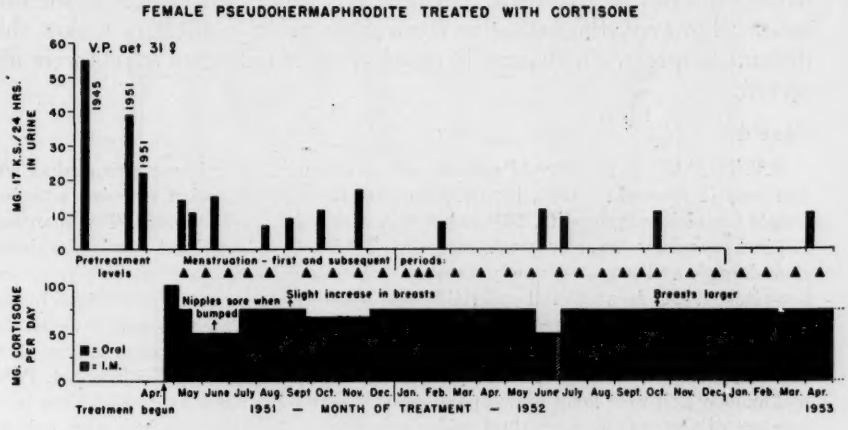

FEMALE PSEUDOHERMAPHRODITE TREATED WITH CORTISONE
V.P. aet 31 ♀
MG. 17 K.S./24 HRS. IN URINE
1945
1951
1951
Pretreatment levels
Menstruation - first and subsequent periods
MG. CORTISONE PER DAY
Nipples sore when bumped
Slight increase in breasts
Breasts larger
■ = Oral
■ = I.M.
Apr. Treatment begun
May June July Aug. Sept Oct. Nov. Dec.
Jan. Feb. Mar. Apr. May June July Aug. Sept. Oct. Nov. Dec.
Jan. Feb. Mar. Apr.
1951 — MONTH OF TREATMENT — 1952
1953

CHART IV

and deeply pigmented; the labia minora were absent. The clitoris had been amputated. The urethral orifice was distinct and in normal relation to the vaginal orifice. The uterus and cervix were extremely small, and the ovaries were not palpable. A vaginal smear, stained by the Shorr trichrome technique, showed small, blue-staining epithelial cells with large active nuclei. Although evidence of cornification was lacking, the smear did not show completely atrophic cells. Results of urinalysis, blood count, and a glucose tolerance test were normal. Urinary 17-ketosteroid[10] output was 39.1 and 22 mg. per twenty-four hours. Roentgenograms of the skull showed no abnormalities. An intravenous urogram revealed only an incidental finding of a spina bifida cleft of the twelfth dorsal and first lumbar vertebral bodies.

Treatment. Treatment with cortisone was begun on April 20, 1951 (Chart IV). During oral cortisone therapy, 25 mg. three times daily, the 17-ketosteroid excretion dropped from an average of 30 mg. per twenty-four hours to values varying from 6.85 to 11 mg. (average, 8.9 mg.). On a dosage of from 50 to 67.5 mg. daily, the 17-ketosteroids measured between 11.75 and 17.25 mg. per twenty-four hours (average, 14.7). After a five-

[10] Method of Drekter, Pearson, Bartczak and McGavack (9).

day trial of cortisone intramuscularly, 50 mg. daily, the 17-ketosteroid output was 9.5 mg. The patient found parenteral administration of cortisone painful and voluntarily resumed oral medication, 75 mg. daily.

Over a period of twenty-four months, 48,525 mg. of cortisone orally and 250 mg. of cortisone acetate were administered intramuscularly. The patient is continuing cortisone treatment.

Results. The first true menstrual period occurred three weeks after initiation of therapy. The periods continued to occur fairly regularly; they were ovulatory in type as shown by basal body temperature chart, of three to fours days' duration, but scanty in amount. The breasts developed somewhat (Fig 3b). The patient gained 10 pounds in weight. No facial changes nor changes in blood pressure or carbohydrate metabolism occurred. The patient did not believe there had been any significant change in the hirsutism; however, the extensive electrolysis prior to therapy makes this difficult to assess. No changes in mood or other untoward effects were observed.

Case 4

P.C. (U.C.H.), a 21-year-old woman with a complaint of primary amenorrhea was first seen in December, 1951, by Dr. Charlotte Baer. Examination revealed a typical female pseudohermaphrodite, 56½ inches tall, and weighing 97 pounds. The span was 55½ inches and the lower body measurement, 26¾ inches. The patient was long-waisted, short-legged, and narrow-hipped; mammary development was lacking. The voice was low-pitched. Her face was full and ruddy and her eyes prominent. Her forehead bulged and the temporal hairline receded markedly. There was no apparent acne or facial hirsutism. The chin felt somewhat rough, however, as though hair had been broken off at the skin level or had been removed with an abrasive, which the patient denied. Pubic and axillary hair were luxuriant. A phallus was described as 5 cm. long and 2.5 cm. in its greatest diameter, with a urethral orifice at the base. The labia majora were well developed; labia minora were lacking, and instead a thin curtain of tissue almost covered the vaginal introitus, which was separate from, and posterior to the urethral opening. The vaginal canal measured 3 inches in length. A small cervix with a patulous external os could be seen with a panendoscope.

The family history was noncontributory. The parents were living and well. There was no consanguinity. A brother, aged 25, was normal. The patient had been very tall up to the age of 10, but at 14 she was the shortest of her classmates.

X-ray films of the skull and intravenous pyelograms showed no abnormalities. All the bony epiphyses were fused. Urinary 17-ketosteroid[11] excretion was 292 and 323 mg. per twenty-four hours.

The tentative diagnosis was congenital adrenocortical hyperplasia, but because of the unusually high level of urinary 17-ketosteroids, the possibility of an adrenocortical tumor was entertained. On September 9, 1950, a transabdominal exploratory operation was performed by Dr. Frederick Howard. The ovaries were described as small, the left 3 by 2 cm., and the right 3.5 by 2 cm. Uterus and tubes were infantile. Both adrenals were distinctly enlarged, the right measuring 6 by 3 by .75 cm. and the left 8 by 6 by 1

[11] Method of Drekter, Pearson, Bartczak and McGavack (9).

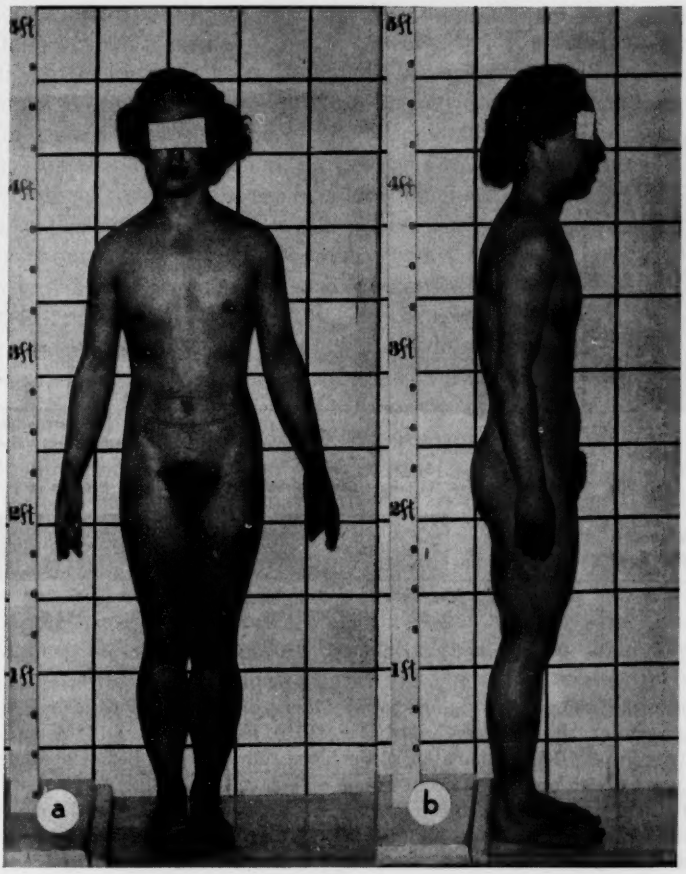


FIG. 4. *Case 4.* Female pseudohermaphrodite, aged 21 years. *a.* and *b.* Note short stature, narrow hips, short legs, and lack of mammary development.

cm. Two-thirds of the left adrenal was removed, and biopsy specimens were taken of both ovaries. Microscopic examination of the latter showed dense ovarian stroma, with numerous small primordial follicles and an occasional graafian follicle. The removed adrenal tissue, plus the adjacent fat, weighed 8 Gm.; considerable fresh hemorrhage was observed. On microscopic examination, the cortex was slightly thickened; most of the increased width seemed to be in the zona reticularis, which contained an unusual amount of pigment. When viewed under the polarizing microscope, normal amounts of dust-like birefringent lipids were seen; however, their presence in the deepest third of the fasciculata was a shift inward from the usual position.

One month after partial adrenalectomy, the urinary 17-ketosteroid excretion had dropped to 122 mg. per twenty-four hours, and a year later to 84.5 mg. On April 3, 1951, a clitoridectomy and plastic repair of the vagina were performed.

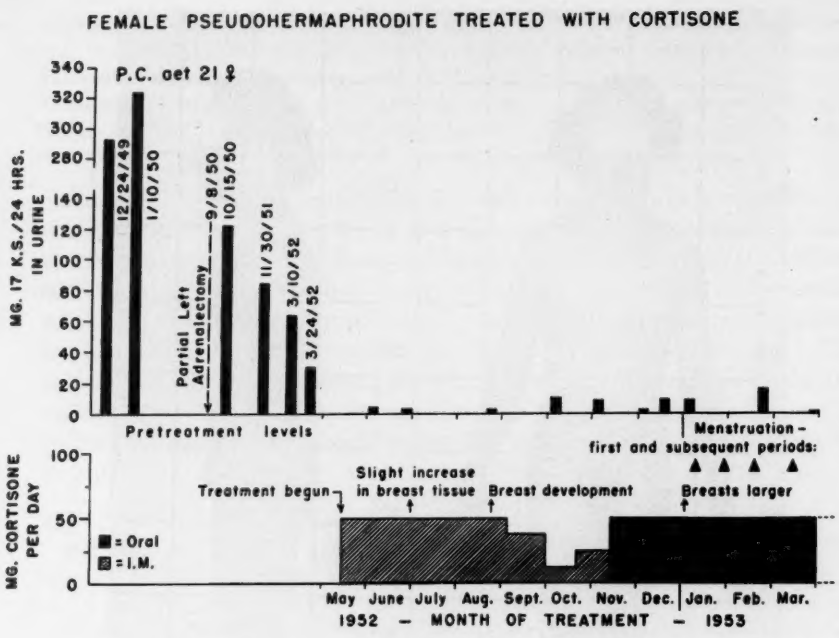


CHART V

On December 6, the patient was first seen by the author. Except for the evidences of surgical intervention, she was as described (Fig. 4a, b). Her weight was 101 pounds, and the blood pressure 120/80. Examination of the blood showed a high hemoglobin content (16.4 Gm.) and a high erythrocyte count (5.65 million cells per cu. mm.) for a woman. Results of urinalysis and a glucose tolerance test were normal. The 24-hour urinary 17-ketosteroids[12] measured 63.8 on March 10, 1952, and 30 mg. on March 24, at which time the corticosteroids[13] in the urine were reported as 3.3 mg. per twenty-four hours (normal 5 to 15 mg.).

Treatment. Treatment with cortisone acetate, 50 mg. intramuscularly daily, was begun on May 13. Chart V shows the correlation of dosage and 17-ketosteroid output. A satisfactory dosage proved to be 50 mg. every other day. After six months of parenteral cortisone there were no subjective or objective changes other than a reduction in urinary 17-ketosteroid output and a slight growth of mammary tissue. For purposes of comparison, the treatment was changed on November 13 to 50 mg. of cortisone orally, daily, given as follows: 12.5 mg., 12.5 mg., and 25 mg. at eight-hour intervals. The 17-ketosteroid excretion remained at approximately 9 mg. per twenty-four hours; the corticosteroids rose to 27.8 mg. The breasts continued to develop slowly, and the patient experienced her first menstrual period on January 9, 1953. Subsequent periods followed at fairly regular intervals.

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[12] Method of Callow, Callow and Emmens (16).

[13] Method of Reddy, Jenkins and Thorn (17).

Summary. A 22-year-old female pseudohermaphrodite, with an extraordinarily high output of urinary 17-ketosteroids for this condition, showed remarkably little hypertrichosis. Following a partial left adrenalectomy, the output of 17-ketosteroids steadily diminished. It seems incredible that removal of a portion of one hyperplastic adrenal alone should account for this result. A possible explanation, on the basis of the considerable degree of acute hemorrhage seen in the removed portion of the adrenal, is that total infarction or destruction of the left adrenal may have ensued.

Twenty months after partial adrenalectomy, the patient's physical condition had not changed. Cortisone therapy was then begun. Within eight months, after a total of 7,575 mg. of cortisone intramuscularly and 2,500 mg. orally, the patient's breasts had developed and menstrual function had been initiated. No significant changes were noted in weight, facies or blood pressure. No glycosuria was detected. The only change, other than those of feminization, was one of mood. Whereas originally the patient had been most uncommunicative, answering questions barely in grunts, under treatment she became first monosyllabic in her answers and later more talkative and pleasant. Treatment is continuing.

DISCUSSION

In discussing the physiopathology of congenital adrenocortical hyperplasia, emphasis should be placed on the fact that this form of the disease is totally different from the acquired variety. The latter consists usually of a mixture in varying proportions of the adrenogenital and Cushing syndromes. The former is that of pure virilization, commencing in prenatal life. It should be noted that whereas the urinary 17-ketosteroid excretion was invariably elevated, the 17-hydroxycorticosteroid content of blood and/or urine was abnormally low in the 3 cases in which it was determined. Kelley, Ely and Raile (5) observed the same phenomenon in 3 of their cases. These findings are correlated very well with the currently developing concept of the disease (4, 5), which may be stated as follows:

1. A congenital abnormality of the adrenal cortex renders it incapable of elaborating a normal amount of carbohydrate-regulating steroids (18).

2. As a consequence, the anterior hypophysis is stimulated to produce corticotropin in excess (as gonadotropins are produced in excess in primary gonadal deficiencies).

3. The adrenals respond to this excessive corticotropic stimulation in the only way in which they are capable, namely by pouring out a superabundance of androgens, which appear in the urine as 17-ketosteroids.

4. This excessive outpouring of androgens results in the virilization which characterizes the disease.

5. When exogenous cortisone is supplied to the patient in adequate

amount, the pituitary corticotropin output is depressed and the output of adrenal androgens falls.

From the scant evidence afforded by such a small number of cases, it would appear that cortisone therapy might be considered to be substitution therapy for a deficiency of the carbohydrate-regulating factors. If this is true, then like all substitution therapy it probably must be continued indefinitely. Certainly seventeen months of treatment resulted in no permanent effects in Case 1.

The question may also be asked as to what part, if any, partial or subtotal adrenalectomy should play in the treatment of congenital adrenocortical hyperplasia. It is interesting to note that in Cases 3 and 4, the urinary 17-ketosteroid excretion was substantially lower following partial adrenalectomy than before. Does this mean that it might be advisable in some cases to precede cortisone therapy with partial or subtotal adrenalectomy? It remains for future controlled experience to provide the answer to this question.

SUMMARY AND CONCLUSIONS

Four patients with congenital adrenocortical hyperplasia were treated with cortisone, 1 male aged 6 years, and 3 female pseudohermaphrodites aged 13, 21 and 31 years, respectively.

In the 3 female patients, cortisone given orally, 50 to 75 mg. daily in divided doses, maintained a satisfactory inhibition of 17-ketosteroid output, with the result that breasts developed and menstruation occurred. The effect on hirsutism was difficult to appraise, since one patient had had electrolysis and another was not excessively hirsute. The hair growth in the third patient was somewhat attenuated.

Optimum suppression of 17-ketosteroid excretion in the one male patient was not achieved on the dosage schedule used. Dosage was inadequate over a considerable period of time and was not properly distributed over the twenty-four hours of the day. More than 50 mg. daily, orally, was not well tolerated. Nevertheless, a satisfactory result was achieved in inhibiting skeletal maturation. The importance of this lies in the fact that eventual dwarfing was avoided in this boy. According to his present height and bone age, he should achieve normal height.

Untoward results were the development of *duodenal ulcer* in the male patient and of a *schizoid episode* in the youngest female. Both patients recovered completely within three months after cessation of therapy.

Sudden withdrawal of cortisone in 2 patients was followed by intense asthenia suggestive of adrenocortical insufficiency. Although recovery took place after two to three weeks, the asthenic episodes suggest that sudden withdrawal is to be avoided if possible, and that a short period of corti-

cotropic stimulation might be desirable before cortisone is discontinued entirely.

Therapy for as long as seventeen months effected no permanent inhibition of endogenous corticotropin production nor of adrenocortical reactivity. It is suggested that cortisone therapy for adrenocortical hyperplasia of the congenital variety is in fact substitution therapy for a deficiency of the carbohydrate-regulating function of the adrenal cortex. As substitution therapy, it probably must be continued indefinitely, unless experience with more prolonged continuous therapy proves otherwise. Obviously, experience with many more cases over much longer periods of time is necessary for final evaluation of the merits of cortisone therapy, with or without subtotal adrenalectomy, in the treatment of congenital adrenocortical hyperplasia.

Acknowledgments

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STUDIES ON THE METABOLISM OF ADRENAL STEROIDS IN THE ADRENOGENITAL SYNDROME*

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THE excessive production of androgenic steroids by the adrenal cortex in the adrenogenital syndrome due to adrenocortical hyperplasia and the clinical manifestations of this disordered function have been well documented (1). The basic derangement of adrenocortical function in this disease awaits precise clarification. Bartter *et al.* (2) suggested that the adrenal cortex was unable to synthesize readily glucogenic corticoids (compound F) so that the pituitary secreted excessive quantities of adrenocorticotropic hormone, which then over-stimulated the target organ in an attempt to attain a "normal" level of such steroids. This thesis would find support in the successful suppression of 17-ketosteroid secretion by the administration of cortisone according to Wilkins (1).

In a preliminary report (3) it was indicated that the excretion of material measured as 3-alpha-20-alpha-pregnanediol (hereafter referred to as pregnanediol) was regularly high in the adrenogenital syndrome and that the levels fell following the institution of therapy with cortisone. Evidence was presented to show that the steroids so measured were in great part 3-alpha-17-alpha-20-alpha-pregnanetriol (hereafter referred to as pregnanetriol), and a simple screening procedure was described for its detection. The present studies were undertaken in order to shed more light on the fundamental causes of the derangement in hormonal synthesis in this condition.

METHODS

Twenty-four-hour collections of urine were preserved with toluene and stored at 4° C.

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In most instances these urines were extracted within a week of collection; several specimens were extracted after six to twelve months (1) of storage.

The urinary 17-ketosteroids were determined by the modified method of Jensen (4). Pregnanediol was measured by the method of Sommerville and Marrian (5) with modifications noted in an earlier communication (3), so that it might be utilized as a screening device for the possible presence of pregnanetriol. The level of 17-hydroxycorticoids in the blood was estimated by the method of Nelson and Samuels (6).

Nonketonic steroids were chromatographed on alumina and eluted with mixtures of ethanol in benzene, according to Mason (7). The specific absorption pattern for the identification of pregnanetriol in sulfuric acid is shown in Figure 1 and has been presented in greater detail elsewhere (3). Also of aid in the identification of the latter steroid, was the development of an evanescent rose color when water was rapidly added to this

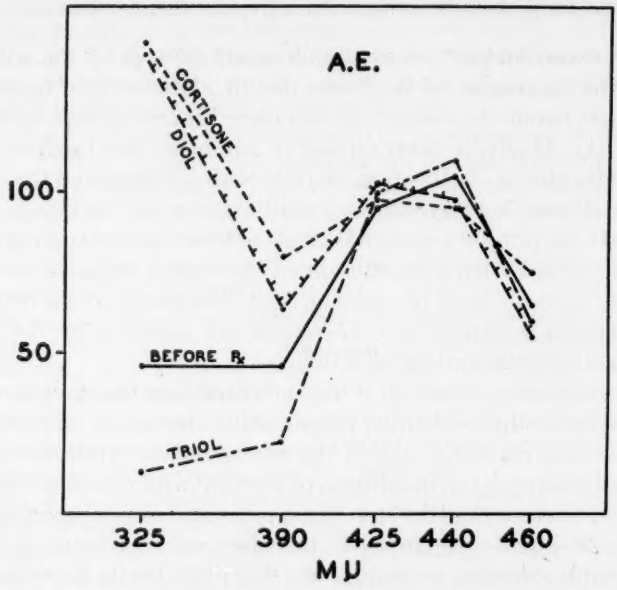

FIG. 1. The spectroscopic patterns of the sulfuric acid spectra of the urine extracts are shown for a single patient (AE) before and after suppressive therapy with cortisone. These are compared with the patterns for pregnanediol and pregnanetriol. The curves are plotted as percentage absorption with reference to the absorption at 425 mμ.

compound in sulfuric acid; this phenomenon was at no time observed with other compounds. Attention has been called to the absorption maximum at 440 mμ for pregnanetriol as contrasted to 425 mμ for pregnanediol. This peak, in addition to differences in the spectrum below 400 mμ formed the basis for differentiating the two steroids. The coefficient of extinction for pregnanetriol at 425 to 440 mμ is about six times that for pregnanediol, so that small quantities of the former would be expected to contribute greatly to the absorption of light in this region when measuring mixtures of the two substances, as may occur when using the method of Sommerville (5) for pregnanediol. It was found that as little as 7 per cent of pregnanetriol in a mixture, would result in an

optical density at 440 equal to that at 425. Larger quantities will introduce a peak at 440 mμ. Various formulae were devised for the determination of the quantity of each substance in a mixture, based on the differences in light absorption at various wavelengths. Although these were applicable to a mixture of pure compounds, they were grossly inaccurate when applied to extracts of urine, because of the presence of interfering impurities. For this reason it was not possible to state precisely the quantity of each compound when both were present, and for the sake of conformity the results, other than those of chromatographic separation, are expressed as "pregnanediol" although the spectra to be described were indicative of the presence of large quantities of pregnanetriol in certain specimens. The measurements were made with a Beckman DU quartz spectrophotometer.

Because of the interest in intermediary steroid metabolites, particularly with reference to 17-hydroxyprogesterone, studies were performed to find a specific test for the latter substance. It was fortuitously discovered that 17-hydroxyprogesterone reacted in the so-called "blue test" of Allen (8) which was designed for the estimation of dehydroepiandrosterone. The absorption spectra were identical, with the exception that the spectrum for 17-hydroxyprogesterone had a coefficient of extinction at 600 mμ which was about one fifth that of dehydroepiandrosterone. Certain eluates from the Florosil columns for the determination of blood corticoids according to Nelson and Samuels (6) were subjected to this test in order to detect 17-hydroxyprogesterone, although it was appreciated that a positive result would not have been specific. Preliminary experiments showed that 17-hydroxyprogesterone could be quantitatively eluted with 2 per cent methanol in chloroform.

RESULTS

Excretion of pregnanetriol and pregnanediol

Table 1 summarizes the levels of "pregnanediol" in the urines of 13 patients with the adrenogenital syndrome due to adrenocortical hyperplasia, as defined by Wilkins (1). In addition, normal values are included for adults and children, as well as for patients with hirsutism, Cushing's syndrome and virilizing adrenocortical tumors. The levels in cases of adrenocortical hyperplasia were uniformly elevated and regularly suppressed upon the administration of cortisone. These data must be carefully qualified. Since the method employed was designed to measure pregnanediol, the figures were calculated as the quantity of material present, based on the extinction coefficient of this steroid. In general, the spectroscopic patterns of the final color conformed to those of pregnanediol, but in every instance of the adrenogenital syndrome prior to therapy, the absorption spectra resembled those of pregnanetriol. This is illustrated in Figure 1, in which the curves for pregnanediol and pregnanetriol are compared with those for the urine extract from a patient with the adrenogenital syndrome, before and during treatment with cortisone. The justification for assuming that the material measured before treatment was primarily pregnanetriol has been detailed earlier (3). Chromatographic data presented here will illustrate the basis for this conclusion. In Table 1, results expressed in italics

TABLE 1

Patient	Sex	Age	Diagnosis	17-KS before treat. (median)	Urinary "pregnanediol"* (mg./24 hrs.)	
					Before cortisone	After cortisone
AB	M	31	Normal	9	0.1 –0.29	
HS	M	33	Normal	7	0.15	
MT	F	29	Normal		0.4 –15.0	
JW	M	4	Normal		0.05	
MC	F	4	Normal		0	
CB	F	4	Normal		0.02	
BW	M	3	Nephrosis		0.03	
MC	M	3	Nephrosis		0	
BR	M	16	Gynecomastia		3.3	
MA	F	29	Sterility		0.4	
SD	F	32	Sterility		0.9	
PE	F	20	Constitutional hirsutism		1.65	
SD	F	32	Constitutional hirsutism		0.4	
BC	M	3 wks.	Congenital		*2.9*	
MCh	F	7 mos.	adrenal	2.1	*10.3*	
LP	F	3	hyperplasia	8.6	*4.0– 6.8*	0.42 (1 wk.)
FF	F	3		3.4	*15.5–19.8*	0 (2 wks.)
TT	F	3		15.0	*4.9*	1.5 (1 wk.)
CA	F	4		24	*3.8– 7.9*	0.2 (1 wk.)
SB	M	4		12.7	*38.6*	6.9 (3 dys.)
						1.6 (10 dys.)
WD	M	6		19	*13.9*	0.2 (1 mo.)
AE	M	6		5.6	*15.5–18.5*	5.4 (2 dys.)
						0.4 (1 wk.)
DB	M	8		18	*2.4*	0.6 (10 dys.)
LG	M	13		24	*13–45*	3.3 (1 wk.)
						0 (10 dys.)
NW	F	16		26	*37–118*	1.8–3.3 (1 wk.)
						0.1 (1 mo.)
HW	F	30		29	*31.0–53.2*	0.2–0.3 (1 wk.)
MD	F	45	Cushing's syndrome		0.1	
LD	F	38			0.7	
HB	M	5	Adrenal tumor	194.0	135.7	
MC	F	32	Recurrent adrenal tumor	21.0	4.7	

* The results indicated in italics revealed spectroscopic patterns of pregnanetriol in the final color reactions, as qualified in the text.

indicate that the material present showed a peak at 440 mμ, as well as relationships at 390 similar to those of pregnanetriol (Fig. 1). The curves reverted to those corresponding to pregnanediol following the institution of therapy with cortisone (Fig. 1).

TABLE 2. CHROMATOGRAPHY OF NONKETONIC FRACTIONS

Patient	Age	Hydrolysis	Pregnanediol mg./day	Pregnanetriol mg./day
		Adrenal hyperplasia		
LP (before treat.)	3	Acid	0	6.1
LP (cortisone)		Acid	0	0
WD (before treat.)	6	Acid	0.2	3.9
FF (before treat.)	3	Glucuronidase	0	0.9
FF (cortisone)		Glucuronidase	0	0
FF*		Glucuronidase	0	7.1
HW (before treat.)	30	Acid	0.6	7.1
MC normal female	25	Acid	9.2	0
AB normal male	31	Acid	0	0
JC* normal male	4	Acid	0	0

* After the administration of 25 mg. of 17-hydroxyprogesterone (24-hour urine only).

Table 2 summarizes the results of steroid chromatography of the nonketonic fractions from the urines of several patients. These include conjugates hydrolyzed by acid or glucuronidase. The fractions were identified by melting point, sulfuric acid spectra, and point of elution. The identity of one representative residue from the pregnanetriol fractions was confirmed by infrared spectroscopy, through the kindness of Dr. Harold Mason. It would appear from these separations that, in fact, most of the material present was pregnanetriol, with little or no prenanediol. A simple method for separation and quantitative estimation of each steroid has been developed, and will be published later (9). The results with this method support the foregoing data.

Responses of urine and blood steroids to adrenocorticotropin

Several patients with the adrenogenital syndrome were treated with 0.5 mg. of adrenocorticotropic hormone (ACTHAR-C, Armour) per kilogram of body weight, intravenously, over a period of from three to four hours. Table 3 summarizes the excretion of "pregnanediol" in the urine following this stimulation. The data are subject to the same qualification as for Table 1. The increased excretion of "pregnanediol" in patients with the

TABLE 3. ACTH ADMINISTRATION

Patient	Diagnosis	Status	Control		ACTH	
			17-KS mg./day	"P'diol"* mg./day	17-KS mg./day	"P'diol"* mg./day
CA	AH†	Untreated	10–20	*3.8*	33	*5.3*
SB	AH	Untreated	9–14	*30.0*	21	*35.0*
AE	AH	Untreated	6–12	*18.4*	12	*56.0*
NW	AH	Untreated	50–60	*60–110*	54.3	*245.0*
NR	AH	Treat. recently omitted	3–9	1.0	14	*27.8*
FF	AH	Treat. recently omitted	3–6	*2–3*	6.5	*31.0*
LP	AH	Treat. recently omitted	2–5	2–5	6.5	*13.7*
PL	Leukemia			0		1.9
JM	Leukemia			0.5		1.3
CN	Rh. fever			1.4		1.4

* "P'diol" designates 3-alpha-20-alpha-pregnanediol. The figures in italics indicate characteristics of pregnanetriol as noted in the text.

† AH = Adrenal hyperplasia.

syndrome was generally great but was not necessarily attended by a proportional rise in the excretion of 17-ketosteroids for the short experimental period employed. Most remarkable were the rises noted in 2 patients (NR and FF) exhibiting suppression from cortisone therapy which had been discontinued shortly before the experiment. There was restoration of the patterns of urinary residues to those resembling pregnanetriol. Although slight rises were observed in 3 patients with presumably normal adrenocortical function (PL, JM and CN), none of the characteristics of pregnanetriol appeared in the pattern of the urinary residue following adrenocorticotropin.

The blood levels of 17-hydroxycorticoids were determined in 9 patients with the adrenogenital syndrome, before and after similar periods of stimulation with adrenocorticotropin; these are illustrated in Figure 2. They are compared with those of several normal children and adults and of 2 patients with Addison's disease. Among 9 patients with adrenocortical hyperplasia so studied, 7 revealed abnormally low levels initially, which were less than 1 gamma per 100 ml. of plasma. Of the 9 so stimulated, all but 2 failed to show a significant rise in the blood levels of corticoids. There was no relationship between the response of the blood corticoids and the excretion of "pregnanediol." None of the specimens of blood revealed the presence of 17-hydroxyprogesterone before or after stimulation with adreno-

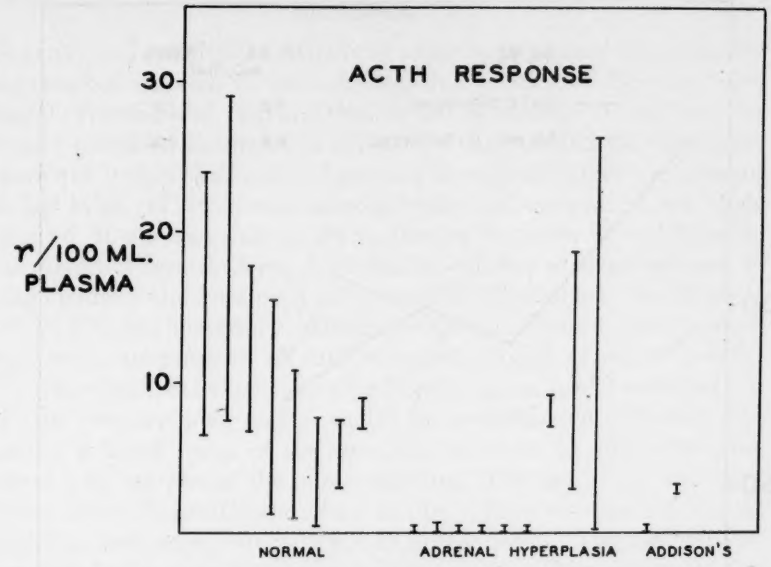

FIG. 2. The lower end of each bar represents the control level of blood corticoids and the upper end, the level after the administration of adrenocorticotropin. The normals include patients with presumably intact adrenocortical function. The results for 2 patients with Addison's disease are shown for comparison.

corticotropin. The method employed would not be expected to detect quantities less than 20 gamma per 100 ml. of plasma.

Administration of 17-hydroxyprogesterone

Several patients were given single intramuscular injections of from 25 to 100 mg. of 17-hydroxyprogesterone and the urinary levels of pregnanediol and pregnanetriol were measured. In most instances the presence of pregnanetriol was detected by the screening procedure described. Figure 3 illustrates the response in Patient HW, who was receiving cortisone therapy and appeared to show suppression of her disease, as indicated by the lower level of urinary 17-ketosteroids and pregnanetriol. The illustration indicates that the urinary steroid spectroscopic pattern of HW, under cortisone therapy, was more typical of pregnanediol and was without evidence of pregnanetriol. Although the increase in urinary "pregnanediol" was not great during administration of 17-hydroxyprogesterone, it is remarkable that the spectroscopic pattern returned to that of pregnanetriol. This change in pattern resembles the responses to adrenocorticotropin described previously and indicates the reappearance of pregnanetriol following the administration of 17-hydroxyprogesterone. Another patient

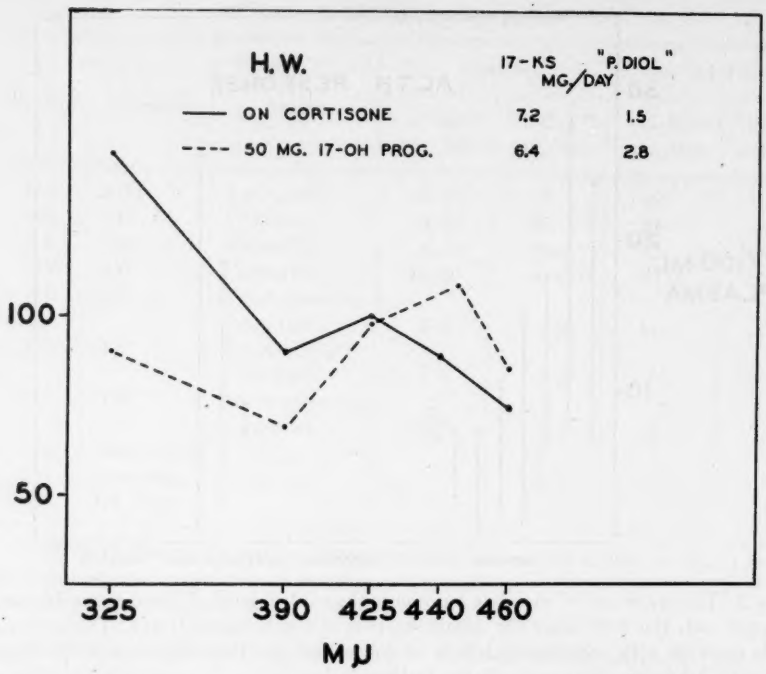

FIG. 3. The absorption curves for urinary extracts from Patient HW while receiving suppressive therapy with cortisone and following the administration of 17-hydroxyprogesterone. These are to be compared with the curves in Figure 1, to indicate the reversal from pregnanediol to pregnanetriol. The quantities of materials expressed as "pregnanediol" are to be interpreted in the light of the qualifications in the text.

(NW) was injected with 100 mg. of 17-hydroxyprogesterone early in the course of cortisone therapy, when the urinary pattern still indicated the presence of pregnanetriol. Material measured as "pregnanediol" rose from a level of 13.1 mg. per day to 58.8 mg. in the 24-hour period following the administration of this steroid; in this instance the excretion of 17-ketosteroids rose from 11.9 to 18.3 mg. per day. A third patient with the syndrome (FF, Table 2) was given 25 mg. of 17-hydroxyprogesterone after the steroid excretion had been well suppressed with cortisone therapy. Detailed chromatographic study revealed an excretion of 7.1 mg. of pregnanetriol in the ensuing 24-hour period, whereas it had been absent previously.

Two normal adults and 2 children with rheumatic fever (who were receiving cortisone therapy) were treated with from 25 to 50 mg. of 17-hydroxyprogesterone. In none of these cases did pregnanetriol appear in the urine.

DISCUSSION

This study was undertaken initially in order to determine the quantities of pregnanediol excreted in the adrenogenital syndrome. Previous data were scant. Venning *et al.* (10) and Genitis and Bronstein (11) reported the presence of increased quantities in a total of 4 patients, but the clinical details were not always clear and in 1 instance an adrenal tumor was present. Stover and Pratt (12) reported that no pregnanediol was present in a single case studied. In our experiments the method of Sommerville and Marrian (5) was preferred because it required smaller volumes of urine, an asset in studying children, and because it had proved to be reliable in the hands of its authors. Clinical laboratory methods for the quantitative measurement of pregnanediol are probably all subject to the criticism of lack of specificity. For this reason, the final color developed in sulfuric acid was read not only at the recommended maximum (5) for pregnanediol (425 mμ), but throughout a broad range of the spectrum, in order to guarantee close agreement with samples of the pure compound. This led to the discovery that the patterns obtained in the adrenogenital syndrome corresponded not to those of pregnanediol, but to those of pregnanetriol. The method was therefore applied as a screening test for the presence of pregnanetriol (3); there was good agreement between these results and those of more detailed chromatographic analysis. Furthermore, the sulfuric acid spectra of other steroids associated with the adrenogenital syndrome were not found to show similar patterns. There were some similarities between pregnanetriol (3-alpha-17alpha-20-alpha pregnanetriol) and certain of the 3-beta compounds (3-beta-17-alpha-20-beta-pregnanetriol and 3-beta-17-alpha-20-alpha-pregnanetriol) (13) but digitonin precipitation did not yield detectable quantities of 3-beta isomers with our material. Since it had not been possible to express accurately the quantities of pregnanetriol present with the method employed, it was believed better to designate the results in terms of "pregnanediol."[1]

The fortuitous discovery that 17-hydroxyprogesterone produces a reaction with the Allen "blue test" identical to that of dehydroepiandrosterone, albeit with a lower coefficient of extinction, makes possible the use

[1] As has been demonstrated, the final sulfuric acid spectra were those of pregnanetriol. A method is currently being employed (9) which will enable a precise estimation of both substances in urine extracts. In this report, only the results of chromatographic separation may be interpreted with finality as to the exact amounts of pregnanediol and pregnanetriol present. The use of the method of Sommerville and Marrian as herein employed is, nevertheless, a helpful guide in the diagnosis and treatment of the syndrome, since it measures large quantities of chromogens which are probably for the most part pregnanetriol, and the nature of the spectral curve is distinctive when readings are taken at 390, 425, 440 and 460 mμ (Fig. 1).

of this test for the detection, but not absolute identification of this compound.

Butler and Marrian (14) described the presence of pregnanetriol in the urine of 2 women with the adrenogenital syndrome, but the steroid disappeared from the urine of 1 patient after unilateral adrenalectomy. One of our patients (HW) showed pregnanetriol in the urine several years after removal of three-fourths of the adrenal glands. Mason and Kepler (7) subsequently isolated the same compound from the urines of 3 women with pseudohemaphrodism and adrenal hyperplasia, and from 1 patient with an adrenocortical tumor, although the latter diagnosis was not confirmed by exploration. Miller and Dorfman (15) also discovered this compound in significant quantity in the urine of a "young woman with masculinization." Although the clinical data in these reports are brief, undoubtedly some of the patients had the adrenogenital syndrome due to adrenocortical hyperplasia as defined by Wilkins (1).

Our findings permit certain conclusions regarding the excretion of pregnanetriol on this condition. Pregnanetriol is regularly present in the urine of untreated patients and is suppressed with adequate cortisone therapy. It rapidly reappears following the short-term administration of adrenocorticotropin or the administration of 17-hydroxyprogesterone. These procedures do not elicit such a response in individuals with normal adrenocortical function.

Whether or not pregnanetriol is "the" steroid metabolite pathognomonic of adrenocortical hyperplasia, is an argument which the authors cannot settle. Certainly its detection in every case of adrenocortical hyperplasia studied, suggests an important relationship to this condition. By using the screening device described previously (3), another diagnostic tool has been added. Two patients (MCh and AE) who on clinical grounds were believed to have this condition, at first excreted quantities of 17-ketosteroids which were not so high as to establish the diagnosis clearly. However, in both, the presence of pregnanetriol assisted in defining the disease, which in one instance (AE, Table 1) was difficult to differentiate from constitutional precocious puberty. Prolonged study eventually revealed a significant elevation in the excretion of 17-ketosteroids in the latter.

It is tempting to speculate on the implications of the foregoing findings. Bartter (2) proposed a defect in glucogenic corticoid synthesis by the adrenal gland in the adrenogenital syndrome, which would effect an increased production of adrenocorticotropin by the pituitary, due to the low level of these steroids. Sydnor *et al.* (16) have shown that the blood levels of adrenocorticotropin are high in this disease. With this effort to restore a normal level of steroid production, the adrenal cortex would synthesize increased quantities of virilizing steroids, thus accounting for the high excre-

tion of 17-ketosteroids and the masculinization associated with this condition. In 7 of 9 patients we studied, the initial levels of blood corticoids were in fact extremely low and in 7 of 9 the response to the administration of exogenous adrenocorticotropin was insignificant. However, in 2 instances a significant rise in circulating corticoids was observed after adrenocorticotropin, suggesting that the defect in corticoid synthesis need not be an "all or none" disorder and that with adequate stimulation a partial block may be overcome. This may be expressed in varying degrees of clinical severity and in varying elevations of the 17-ketosteroid excretion. In the present series, however, no correlation could be drawn between the excretion of pregnanetriol and the response of the blood corticoids to adrenocorticotropin. There are some contradictions to this line of thought. Symptoms suggestive of deficiency of the glucogenic corticoids are the exception rather than the rule in the adrenogenital syndrome (1). Many studies of the urinary corticoids in the syndrome, performed in this laboratory by various methods, have not revealed significantly low levels. These observations may be reconciled to the theory by the suggestion that the overactivity of the pituitary can partially overcome the block in corticoid synthesis (2).

Our studies offer some additional information on the defect in biosynthesis of the corticoids in the adrenogenital syndrome. Hechter (17) and co-workers have proposed a schema for the biosynthesis of 17-hydroxycorticosterone (compound F) by the adrenal gland. The last step involves the conversion of 17-hydroxyprogesterone into compound F. This is illustrated in part in Figure 4. Other pathways via C19 steroids acting as

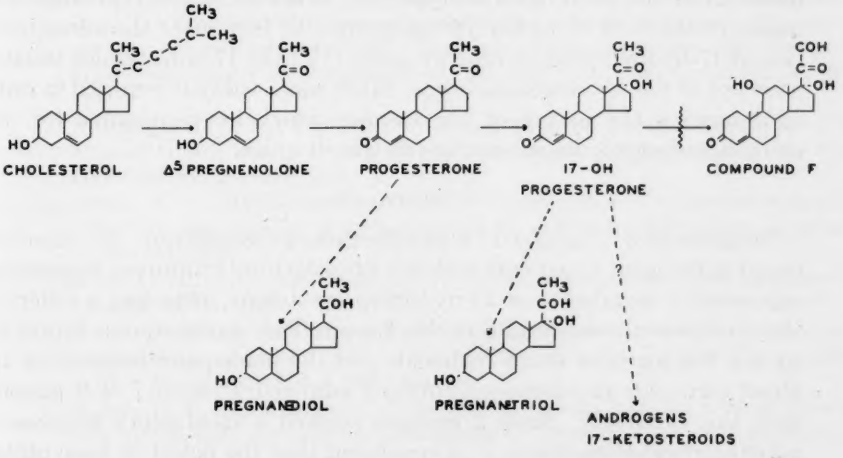

FIGURE 4

acetate receptors are now also known (18). Progesterone is also an intermediary metabolite in the synthesis of compound F from cholesterol. Pregnanediol has long been known to be a metabolite of progesterone, occurring in the urine of females as a result of progesterone production in the corpus luteum, or in males following the administration of exogenous progesterone. By analogy, Mason (7) has reasoned that pregnanetriol is a metabolite of 17-hydroxyprogesterone. Jailer (19) suggested that in the adrenogenital syndrome there is a block in the conversion of 17-hydroxyprogesterone into compound F. Our studies also suggest that the block is at this point rather than at the point of conversion of progesterone into 17-hydroxyprogesterone, since pregnanetriol is a metabolite of the latter, whereas pregnanediol if formed from progesterone. Furthermore the administration of 17-hydroxyprogesterone to patients with this disease has resulted in the excretion of pregnanetriol; this was not the case in individuals with normal adrenocortical function.

Jailer (19) pointed out that 17-hydroxyprogesterone had considerable androgenic activity and suggested that its "piling up" might be responsible for the masculinizing features of the adrenogenital syndrome. We have been unable to detect 17-hydroxyprogesterone in the blood of our patients. Its reduction to pregnanetriol may be so rapid that it does not appear in detectable quantities in plasma. It is unlikely that pregnanetriol per se is androgenic.

There is no definite knowledge as to whether 17-hydroxyprogesterone is converted into other steroids which have androgenic activity. A large proportion of the 17-ketosteroids in the urine of patients with adrenal hyperplasia are of the 11-oxygenated type (20). It is improbable that these represent products of 17-hydroxyprogesterone. In fact, after the administration of 17-hydroxyprogesterone by Jailer (19), the 17-ketosteroids isolated were not of the 11-oxygenated type. Much more study is required in order to determine the nature of the steroids which are responsible for the marked androgenic manifestations in this disorder.

SUMMARY

Pregnanetriol (3-alpha-17-alpha-20-alpha-pregnanetriol) is regularly found in the urine of patients with the adrenogenital syndrome. It probably represents a metabolite of 17-hydroxyprogesterone, reflecting a defect in the synthesis of compound F in this disease. This explanation is supported by the low levels of blood corticoids and the inadequate response of the blood corticoids to adrenocorticotropin administration in 7 of 9 patients with this syndrome. Since 2 patients showed a satisfactory response to adrenocortical stimulation, it is concluded that the defect in biosynthesis is not complete.

The excretion of pregnanetriol, like that of 17-ketosteroids, is suppressed upon the administration of cortisone. In the adrenogenital syndrome it rapidly reappears following the administration of adrenocorticotropin (for short periods) or of 17-hydroxyprogesterone. Its presence may aid in the diagnosis, when the 17-ketosteroids are low.

Methods have been described for the detection of 17-hydroxyprogesterone and pregnanetriol.

Although the studies shed additional light on the abnormal pathways of steroid synthesis in the adrenogenital syndrome, the nature of the steroids responsible for the marked androgenic manifestations has not yet been determined.

Acknowledgments

We wish to thank Dr. Harold Mason for the sample of pure 3-alpha-17-alpha-20-alpha-pregnanetriol and for his assistance with the infrared analysis. The 17-hydroxyprogesterone was kindly furnished by Dr. L. B. Hobson of Squibb and Sons, New York and the pregnanediol by Dr. H. F. Hailman of the Upjohn Company, Kalamazoo, Michigan. We are indebted to Mrs. Mary Ellen Crafton and Mrs. Betty Lawrence for their faithful technical assistance and to Mrs. Lawrence for preparation of the figures. Some of the material studied was kindly furnished by Doctors Milton Markowitz of the Johns Hopkins Hospital and Wallace W. McCrory of the Children's Hospital of Philadelphia. We acknowledge with pleasure the helpful assistance and advice of Doctors Lawson Wilkins and George Clayton.

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EFFECTS OF INTRAVENOUS ACTH ON BLOOD LEVELS OF 17-HYDROXYCORTICOSTEROIDS AND CIRCULATING LEUKOCYTES*

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ALTHOUGH studies on ACTH are numerous, relatively few attempts have been made to determine the effects of the drug, intravenously administered, on the levels of adrenal steroids in the peripheral blood (1, 2, 3).

In this investigation normal subjects were studied to determine the responsiveness of the adrenal cortex of man to the intravenous injection of different amounts of ACTH, with the hope that such a procedure could be standardized for later use as a test of glandular function in disease. Changes in the levels of 17-hydroxycorticosteroids in the plasma were followed as a measure of glandular activity.

As indicated previously (4), the method of Nelson and Samuels (5) for the determination of 17-hydroxycorticosteroids in blood, although not chemically specific for 17-hydroxycorticosterone (compound F), is a relatively accurate measure of this steroid in man. Since compound F appears to be the chief hormone secreted by the adrenal cortex in man (2, 5), this technique serves as an excellent direct measure of the level of circulating adrenocortical metabolic steroids in the peripheral blood.

Concurrent hematologic studies were included to define the comparative reliability of changes in the eosinophils, lymphocytes and total white blood cells as a measure of adrenocortical function in man.

MATERIALS AND METHODS

All subjects were "normal" male and female adults whose ages were between 20 and 42 years. No subject was studied more frequently than once a week. A standard test

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procedure was followed: 30-ml. blood samples were drawn from fasting subjects at 8:00 A.M., 8:15 A.M., 9:00 A.M., 10:00 A.M., and 12 noon. Blood samples were placed in tubes containing 4 mg. of heparin. These were then centrifuged, the plasma removed, and the samples refrigerated until analyzed. Immediately after the first sample was taken, administration of ACTH was begun. When given as a single injection, the hormone was dissolved in 3 ml. of 0.9 per cent sodium chloride and administered intravenously over a period of from fifteen to thirty seconds. For the one-hour infusions the ACTH was dissolved in 100 ml. of isotonic sodium chloride.

All studies were made with Armour ACTH, using the same lot throughout.[1] Eosinophil counts were performed by the method of Randolph (6). These were carried out singly, except for the control and four-hour samples, in which instance duplicate counts were made and the average taken. The white blood cell counts were determined by conventional techniques, and the absolute number of lymphocytes calculated from these and from a 200-cell differential leukocyte count. The same technician made all blood counts. The 17-hydroxycorticosteroids in the plasma were measured by the procedure of Nelson and Samuels (5).

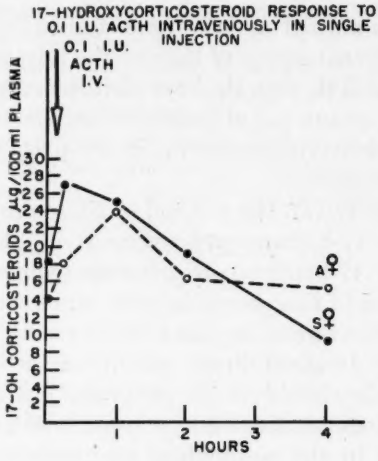

FIGURE 1

RESULTS

Minimal dosage of ACTH necessary to activate the adrenal cortex

The question of the smallest amount of ACTH required to stimulate adrenocortical activity was not explored in complete detail. However, a single intravenous injection of 0.1 I.U. of ACTH induced a brisk and significant rise in the level of blood steroids in the 2 subjects studied (Fig. 1). When 0.1 I.U. of ACTH dissolved in 100 ml. of isotonic sodium chloride

[1] We wish to thank Armour Laboratories, Chicago, Illinois, for generous supplies of ACTHAR, Lot K52905.

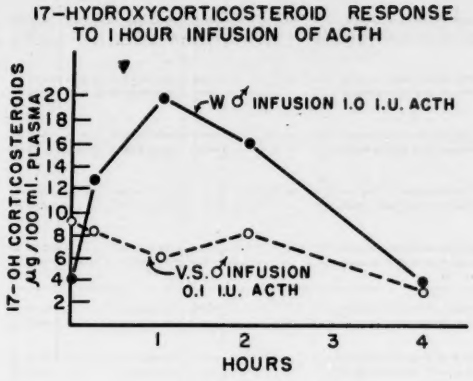

FIGURE 2

was given to one subject by intravenous infusion over a period of one hour, there was no significant effect on the steroid level. Another subject received 1.0 I.U. of ACTH in an identical solution over the same one-hour period; a considerable increase in the concentration of 17-hydroxycorticosteroids followed the large dosage (Fig. 2). It appears that small amounts of ACTH will stimulate the gland to considerable activity, particularly if

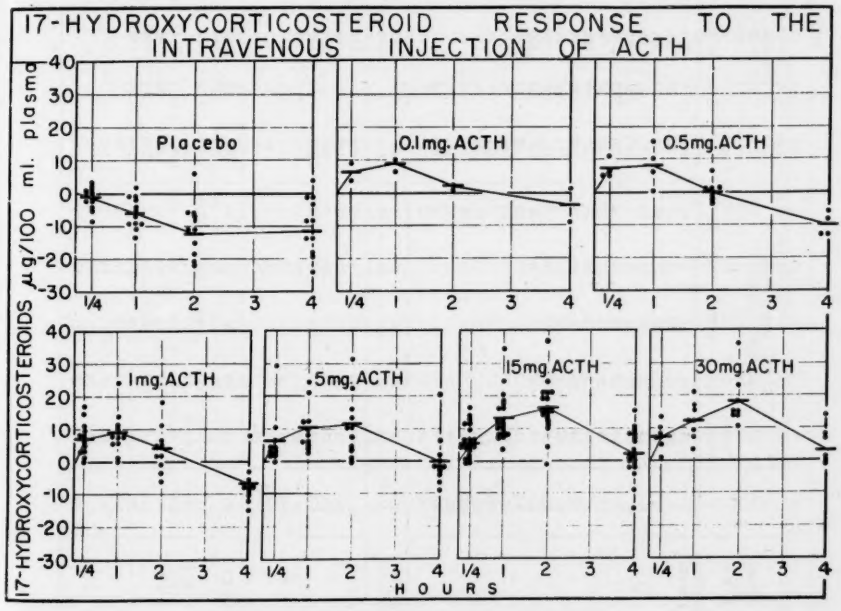

FIG. 3. (1 mg. ACTH = 1 I.U. ACTH.)

TABLE 1

Dosage of i.v. ACTH	Subject	17-OH Corticosteroids (μg./100 ml. plasma)					Eosinophils (per cu. mm.)					Lymphocytes (per cu. mm.)					Leucokytes (per cu. mm.)				
		Control 8 A.M.	8:15	9:00	10:00	12:00	Control 8 A.M.	8:15	9:00	10:00	12:00	Control 8 A.M.	8:15	9:00	10:00	12:00	Control 8 A.M.	8:15	9:00	10:00	12:00
Placebo	Y	13	11	6	6	12	225	175	119	175	119	3.8			2.3	3.6	10.2	8.5	13.0	8.7	8.6
	N	31	26	20	10	7	75	62	56	38	25	2.9	2.7	2.7	2.9	1.9	9.4	8.7	8.5	10.1	8.3
	K	29	21	20	18	15	75	44	62	31	19	2.0			2.0	1.3	6.2	5.8	4.3	5.8	11.4
	R	23	23	22	12	22	62	56	62	44	19	2.7	2.7	2.8	2.6	2.8	8.0	8.3	7.8	7.3	7.6
	S	30	31	21	8		162	131	81	62	56	3.1	1.8	2.7	1.5	2.7	8.4	6.7	9.6	6.6	11.0
	S	26	24	17	11	8	250	231	250	94	125	3.3			3.6	3.0	8.5	9.5	10.4	9.6	9.3
	D	25	26	21	17	17	62	69	50	62	44	2.8			2.6	2.1	8.6	8.0	9.9	9.7	8.5
	A	14	13	12	20	18	81	69	62	81	100	4.5			5.9	4.3	11.8	14.3	12.1	12.5	10.6
	M	26	28	25	20	23	88	75	56	62	88	2.1			3.1	2.1	6.4	8.8	7.2	7.2	7.1
	C	12	8	7			112	106	88	75	100	2.3			2.3	1.2	5.2	4.9	4.8	5.4	5.1
	Mc	20	23	22	14	6	112	150	88	56	44	2.8			3.3	2.4	8.7	8.9	8.8	8.8	9.0
	R	27	27	14	9	8	125	94	131	94	125	2.1			3.3	2.3	7.9	9.2	8.0	8.0	8.5
	Ne	17				10	62				56										
	O	11				14	156				138										
	McC	27				10	231				200										
	Ol	16				12	69				94										
	St	11				12	569				638										
	Ri	8				6	112				144										
	Ro	9				7	138				125										
	Sm	8				7	119				88										
0.1 I.U.	S	18	27	25	19	9	131		125	125	69	2.9			2.5	2.8	6.9		7.7	8.1	8.4
	A	14	18	24	16	15	194		194	188	194	5.1			4.5	4.4	11.2		11.7	11.0	11.3
0.5 I.U.	S	19	30	34	25	13	156		162	156	81	5.1			3.0	2.5	9.4		7.1	10.7	7.9
	D	21	26	31	22	8	88		62	62	31	1.5			1.5	2.0	5.7		5.6	6.6	6.9
	R	13	19	19	12	5	138		150	88	75	2.9			2.3	2.3	7.9		6.4	6.0	6.3
	V.S.	18	23	18	15	5	281		288	238	162	2.6			2.2	2.5	7.8		8.6	6.3	8.5
	T	10			10																
	St	7			8																
	MeA	2			3																
	C	12			10																
1.0 I.U.	Y	19	36	27	24	10	112	106	125	56	38	4.1			2.6	2.6	8.9	8.3	7.5	8.6	10.3
	S	13	14	19	15	5	175	150	188	94	62	2.1			3.2	2.3	8.0	8.7	10.3	12.6	9.9
	D	19	30	34	24	13	81	69	56	38	44	2.4			2.3	2.1	6.6	7.1	6.8	9.5	11.5
	Sc	24	27	25	17																
	A	8	23	32	19	8	231	219	194	125	125	4.3			4.1	4.4	8.8	8.2	10.3	12.3	13.1
	C	22	25	28	16	10	106	88	81	69	19	2.3			1.8	2.4	6.7	6.3	7.0	9.8	9.0
	Mc	18	26	26	19	3	200	181	119	100	75	3.1			5.9	3.9	10.3	10.4	10.2	11.8	12.0
	R	17	23	28	35	9	169	138	138	131	69	2.3			3.4	2.1	6.0	9.3	6.7	7.5	8.9
	Nel	9	17	18	15	6	69			25	25	2.3				1.8	5.5			5.9	8.1
	B	17	26	27	14	7	181			75	44	3.3				2.7	9.1			10.0	13.0
	P	12	12	12	11	4	69			19	12	3.5				3.0	7.6			8.1	14.3
	Sh	13	18	21	15	6	112			12	12	1.8				1.6	5.7			8.4	5.5

TABLE 1—(*continued*)

Dosage of i.v. ACTH	Subject	17-OH Corticosteroids (μg./100 ml. plasma)					Eosinophils (per cu. mm.)					Lymphocytes (per cu. mm.)					Leukocytes (per cu. mm.)				
		Control 8 A.M.	8:15	9:00	10:00	12:00	Control 8 A.M.	8:15	9:00	10:00	12:00	Control 8 A.M.	8:15	9:00	10:00	12:00	Control 8 A.M.	8:15	9:00	10:00	12:00
5 I.U.	Y	12	18	24	28	14	206	62	175	75	31	4.6			2.0	4.1	11.8	10.2	9.6	12.2	12.2
	N	14	16	20	17	10	81	81	69	50	31	4.1			1.4	2.9	9.5	9.3	9.2	10.2	13.7
	K	13	17	19	14	11	69	75	69	75	38	1.9	1.8	1.5	1.3	1.6	5.8	6.1	6.1	6.2	7.1
	R	26	26	35	33	17	69	75	62	44	12	1.8	1.9	1.9	1.1	1.6	6.8	7.6	6.8	6.0	12.6
	S	19	22	27	32	17	112	138	144	69	50	3.4	2.2	2.7	1.2	1.2	8.1	7.6	10.3	8.6	13.1
	D	20	24	32	30	17	81	106	69	38	25	2.2	2.0	1.7	1.9	2.3	7.2	6.7	5.8	8.6	13.3
	D	8	18	32	30	14	100	81	56	81	44	3.2			3.2	2.1	8.2	8.2	8.2	12.5	17.0
	Sh	15	18	20	25	15	112	75	81	25	19	2.8	3.4	2.6	1.8	2.9	7.5	7.8	7.5	6.5	15.0
	A	6	35	27	37	26	88	100	106	62	31	3.7			1.8	2.0	12.5	12.6	13.8	15.2	19.8
	C	15	17	19	15	9	138	94	125	100	38	2.6			2.6	2.4	7.0	6.8	6.5	7.0	9.8
	R	21		24	25	19	162	144	106	56	31	4.5			1.6	1.7	10.6	7.2	7.6	6.4	9.9
15 I.U.	Y			30	33	31	206	150	100	138	38	2.2	3.6	2.1	1.4	2.5	8.8	8.9	7.5	11.9	14.9
	Y	17	18	33	33	15	169	131	162	50	6	3.3	1.6	1.9	1.7	1.7	9.8	8.1	10.3	11.4	15.0
	Y	12	26	20	33	21	138	106	88	94	38	2.8			1.6	1.3	9.3	9.1	8.3	9.3	14.4
	N	19	20	19	31	18	112	125	100	75	50	2.9	3.4	2.3	3.3	2.6	10.4	9.5	9.5	13.5	11.7
	N	14	19	25	29	17	88	81	62	31	12	2.7			2.0	2.2	8.2	8.4	9.3	13.0	13.6
	K	19	22	28	29	21	44	62	56	44	44	2.7	2.2	1.9	1.7	1.5	6.6	6.6	6.2	7.1	9.9
	K	25	29	32	40	25	106	69	75	44	19	1.9	1.8	1.5	1.3	1.6	5.6	5.0	4.6	4.8	8.5
	R	17	21	27	32	8	62	69	69		25										
	R	13	19	21	33	13	69	44	31	12	19	2.6	2.1	1.0	1.1	2.7	6.9	6.5	5.5	8.2	14.8
	S	19	25	31	33	20	175	181	188	112	50	3.2	3.2	3.5	1.8	2.7	8.1	7.8	8.9	9.0	15.8
	S	19	28	26	38	25	200	156	106	106	38	2.8	3.4	1.8	2.0	1.8	8.0	9.8	8.1	9.8	14.8
	D	18	30	38	39	26	75	81	56	44	31	3.2			0.6	0.6	7.7	8.3	6.5	13.3	13.9
	D	13	17	24	26	15	112	56	69	38	12	2.6			1.9	1.2	8.2	7.3	7.9	12.2	14.2
	Sh	21	27	37	32	19	212	150	112	88	25	3.1	4.5	3.2	2.7	3.6	7.5	8.4	6.6	13.9	21.0
	Sh	21	23	28	40	17	175	150	119	69	25	4.2	4.2	2.1	2.0	3.2	9.9	10.1	6.0	10.5	21.1
	Sh	8	24	25	22	23	94	88	69	50	19	2.9	2.5	2.5	2.9	3.3	6.8	6.1	6.7	12.2	19.6
	A	6	18	24	43	7	94	119	56	62	12	1.8			2.3	2.7	5.8	8.0	7.7	16.6	17.6
	A	13	26	37	37	20	100	106	75	44	19	3.7			2.6	1.7	11.1	11.0	9.7	12.5	15.9
	A	18	18	52	39	28	119	112	62	69	31	2.6			1.4	1.9	8.9	9.4	10.6	14.9	18.0
	C	28	28	31	27	13	106	94	138	69	19	3.0			1.3	1.0	7.9	6.4	5.4	6.8	10.0
	R	14	18	25	18	22	112	125	125	50	31	2.8			2.6	1.7	7.5	8.8	7.9	8.8	13.3
30 I.U.	Y	13	20	25	28	21	144	119	94	69	25	2.9			4.5	1.0	8.7	7.7	7.8	17.6	12.0
	R	16	26	25	30	11	31	12	12	38	6	1.6			2.7	2.0	5.7	4.8	5.9	9.9	13.7
	S	17	22	29	35	24	144	119	88	75	19	2.9			1.8	1.1	8.1	7.7	4.5	12.1	14.1
	D	26	33	41	40	27	81	75	56	44	31	1.8			1.8	1.2	8.7	8.5	7.7	13.6	18.0
	Sh	10	23	31	33	24	119	81	125	50	25	1.8			2.6	1.3	5.8	6.4	5.5	10.4	15.1
	A	26		29	62		169	162	112	81	31	4.0			4.0	2.9	10.1	9.1	6.5	16.0	18.1
	C	19	20	26	29	18	144	88	56	31	12	1.4			1.2	1.4	5.6	5.6	5.1	7.9	7.9
	R	7	15	24	22	7	188	194	94	119	56	2.4			1.5	1.0	6.7	6.4	5.0	8.4	12.9

given as a single immediate intravenous injection. This technique was therefore selected for further studies.

The effect of increasing amounts of intravenous ACTH

ACTH was given intravenously by a single immediate injection to a series of normal persons. The dosages used included 0.1 I.U., 0.5 I.U., 1 I.U., 5 I.U., 15 I.U. and 30 I.U.; a placebo was also employed. In some cases it was possible to study the same subjects at almost every dosage level. Other subjects, although interested in the financial remunerations for their services, grew weary of the needle and were unwilling to complete the series. The sequence of administration of the various dosages was scattered, although not completely random. In Figure 3 the composite data are charted, and in Table 1 are summarized. As the amount of ACTH was increased, the mean plasma steroid concentrations two hours after the injection rose until a plateau was reached at the 15-I.U. and 30-I.U. levels. With the exception of one test with 1 I.U., in every case in which an adequate number of samples was drawn, ACTH induced an increase in the concentration of the blood 17-hydroxycorticosteroids.

An increase in the level of steroids in the blood was usually present fifteen minutes following intravenous ACTH, and attained a maximum within one or two hours. The exact form of the curve was related to the size of the dose of ACTH. The greater the amount of ACTH, the higher and more persistent were the elevations in steroid values. With smaller dosages the rises in 17-hydroxycorticosteroid levels were transient, reached a peak earlier and, by four hours, were usually lower than the controls. As illustrated in the curve for placebo injections, the concentration of 17-hydroxycorticosteroids in plasma normally falls during the morning (4).

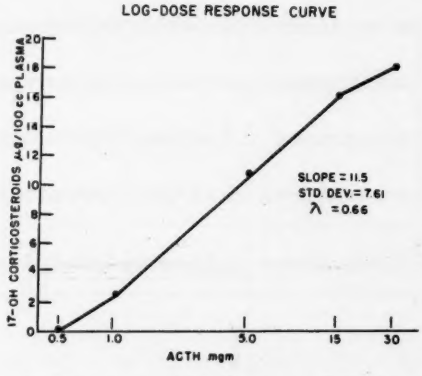

FIG. 4. (1 mg. ACTH = 1 I.U. ACTH.)

Biologic assay of ACTH in man

The data were statistically analyzed to determine whether they fitted a log-dose-response curve. As represented in Figure 4, the logarithm of the dose was charted against the increase or, in a few cases, the drop in the steroid level *two hours* after the introduction of ACTH. As a result of considerable individual variation in the responses of different subjects, the standard deviation was relatively great (7.61). In addition, the slope of the line (11.5), although significant, was not as steep as one might desire for a good biologic assay. As a result, λ was 0.66, an acceptable value for man, but one which falls far short of 0.176 ± 0.016 obtained with the ascorbic acid depletion technique for the assay of ACTH in the rat described by Sayers *et al.* (7).

Individual variations in response to ACTH

There appeared to be a consistency in the magnitude of responsiveness of any single individual to varying amounts of ACTH. Two subjects, in particular, illustrated this point. Subject "C" demonstrated only a slight increase in the concentration of 17-hydroxycorticosteroids at every dosage level of ACTH (Fig. 5). In contrast, the other subject, "A," showed a

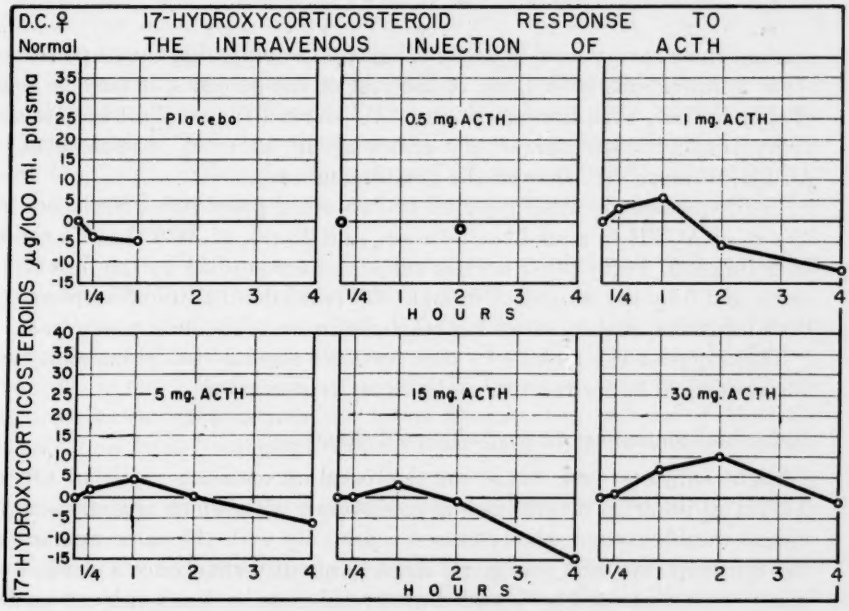

FIG. 5. (1 mg. ACTH = 1 I.U. ACTH.)

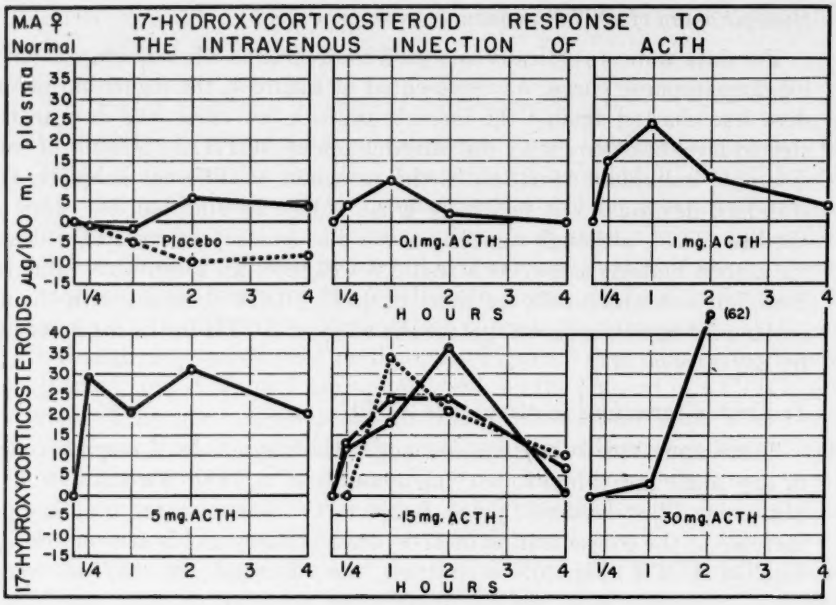

FIG. 6. (1 mg. ACTH = 1 I.U. ACTH.)

marked rise at every level of dosage (Fig. 6). In comparing the responses of these 2 individuals with those of the rest of the subjects, it may be seen (Table 1) that, with few exceptions, "C" showed the smallest increase in 17-hydroxycorticosteroids of the entire group, at every dosage level of ACTH, whereas "A" showed the greatest increase.

The hypo-reactor was subjected to two other procedures. She received 25 I.U. of ACTH in a six-hour infusion, and 25 I.U. of ACTH in an eight-hour infusion. Forty other normal subjects were studied by the first technique and 6 by the second. Although "C" reacted with steroid increases to both infusions, in each series her responses were of the least magnitude.

This suggests the possibility that certain "normal" individuals may be adrenocortical hyper-reactors and others, hypo-reactors.

Individual consistency in level of steroid elevation

Eight subjects were tested on two or more occasions with 15 I.U. of ACTH in order to determine the consistency with which any single individual would respond when stressed repeatedly with the same amount of the hormone. No test was given more frequently than once a week, and many were separated by a period of several months. Each subject tended to respond in a similar fashion, with comparable increases in the concen-

TABLE 2. RESPONSES OF SUBJECTS TO REPEATED TESTS WITH 15 I.U. OF ACTH INTRAVENOUSLY: MAXIMAL INCREASES IN 17-HYDROXYCORTICOSTEROID LEVELS OF PLASMA

Subject	Test 1	Test 2	Test 3
	(μg.)	(μg.)	(μg.)
Y	16	21	
N	12	15	
K	10	15	
R	15	20	
S	14	19	
D	21	13	
Sh	16	19	17
A	37	24	34

tration of 17-hydroxycorticosteroids when retested. The correlation coefficient was .70 (Table 2).

Leukocytic responses

It should be re-emphasized that an increase in plasma 17-hydroxycorticosteroid values occurred after every one of the 57 administrations of ACTH, with 1 exception—a test with 1 I.U. One would expect, therefore, that if the changes in the blood cells reflect adrenocortical function with comparable accuracy, an equally consistent reciprocal response would occur in the eosinophil and lymphocyte counts.

1. *Changes in eosinophils after ACTH.* With 2 exceptions, the eosinophil count decreased 40 per cent or more four hours after the intravenous injections of ACTH, over the range of dosage from 0.5 I.U. to 30 I.U. However the placebo induced a mean drop of 38 per cent, which did not appear to be steroid-determined, since the 17-hydroxycorticosteroid levels fell during this peroid.

The drop in eosinophils was very roughly a graded one, and somewhat proportionate to the amount of ACTH given (Fig. 7).

2. *Changes in lymphocytes after ACTH.* Following the intravenous administration of these small amounts of ACTH, the lymphocytes did not decrease as much as did the eosinophils. In 13 out of 58 tests there was no decrease in the lymphocytes four hours after ACTH. In 8 out of 54 of the tests, when total lymphocyte counts were made both two hours and four hours following ACTH, no drop in lymphocytes had occurred at either point, despite significant elevations in the level of 17-hydroxycorticosteroids.

Four hours following the placebo there was a mean decrease in lymphocytes of 15 per cent, and there was a mean drop of only 25 per cent and 27 per cent after 15 I.U. and 30 I.U. of ACTH. Consequently, although

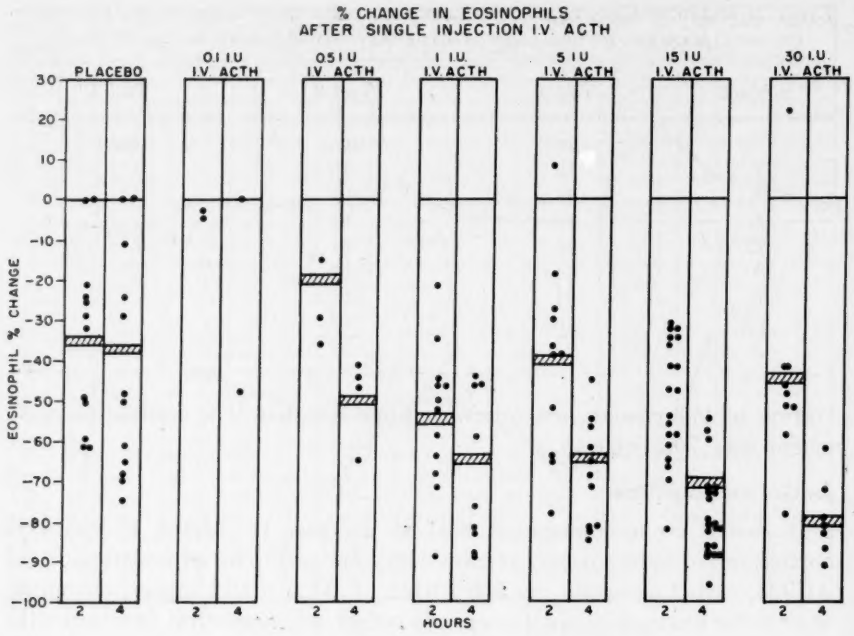

FIGURE 7

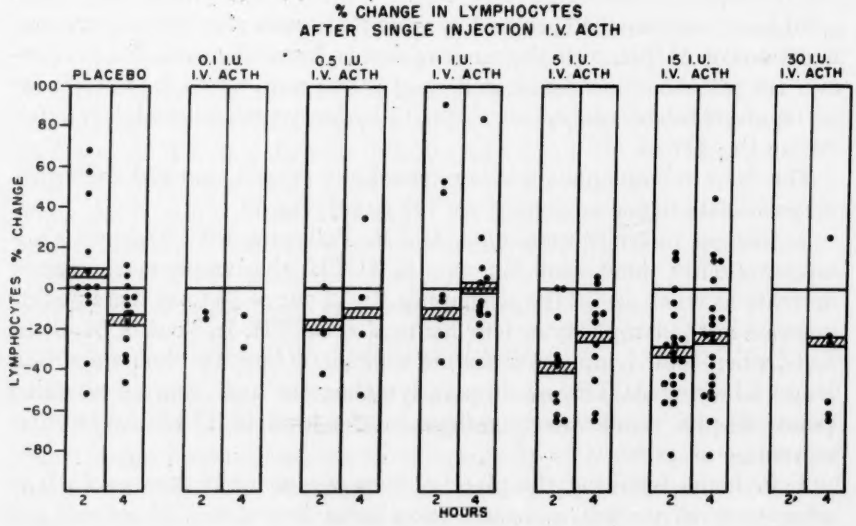

FIGURE 8

group tendencies were in the direction of decreases, individual exceptions were so common and the diminutions were so small that the changes in the numbers of lymphocytes proved relatively unreliable as an indicator of adrenocortical activity (Fig. 8).

3. *Changes in total white blood cell count.* In 6 out of 49 studies, no leukocytosis occurred four hours after ACTH. Unlike the lymphocyte count, however, which showed exceptions at every concentration of ACTH. the total cell count failed to increase only with small amounts of ACTH. All 6 failures occurred with 5 I.U. or less of ACTH (Fig. 9).

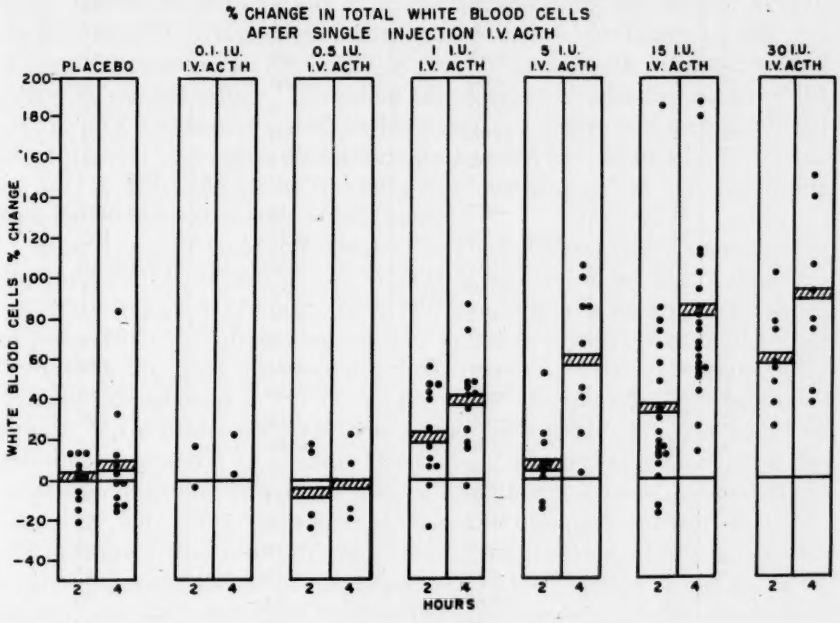

FIGURE 9

4. *Changes in blood cells as a quantitative measure of adrenocortical activity.* A portion of the data was analyzed to determine how accurately the percentage decrease in lymphocytes and eosinophils and the percentage increase in total white cells four hours after the injection of ACTH were a *quantitative* measure of the increase in the steroid concentration of the blood. The elevations in 17-hydroxycorticosteroid levels following 1 I.U. and 15 I.U. of ACTH were tabulated in descending order of magnitude and compared to the percentage changes in lymphocytes, eosinophils and total white blood cells. For example, one subject, "A," had a control steroid level of 6 micrograms per 100 ml. of plasma. Following 15 I.U. of ACTH

TABLE 3. INCREASES IN 17-HYDROXYCORTICOSTEROID LEVELS OF PLASMA IN RELATION TO PERCENTAGE CHANGES IN EOSINOPHILS, LYMPHOCYTES AND LEUKOCYTES

	Increases in 17-OH-corticosteroids (μg.)	Eosinophils (% change)	Lymphocytes (% change)	Leukocytes (% change)
After 1.0 I.U. of ACTH i.v.	24	−46	2	49
	18	−59	− 9	48
	17	−66	−37	16
	15	−46	−12	74
	10	−76	−18	43
	9	−64	−22	47
	8	−62	26	17
	8	−89	−10	−4
	6	−82	4	34
	6	−65	10	24
	0	−83	−14	88
After 15 I.U. of ACTH i.v.	37	−87	44	203
	34	−74	−26	102
	24	−81	−53	43
	21	−59	−81	81
	21	−72	−54	55
	20	−72	4	114
	19	−86	−24	113
	19	−81	−36	85
	17	−80	14	188
	16	−96	−48	53
	16	−88	17	180
	15	−60	−23	60
	15	−82	−16	52
	15	−86	−19	66
	14	−71	−16	95
	13	−89	−54	73
	12	−55	−10	13
	11	−72	−39	77
	10	0	−44	50
	3	−82	−67	27

intravenously, the value rose to 18 micrograms in fifteen minutes, 24 micrograms in one hour, and 43 micrograms in two hours. Thus, the increase in the steroid level was 37 micrograms. At the same time there was an 87 per cent drop in eosinophils, a 44 per cent increase in lymphocytes, and a 203 per cent increase in white blood cells four hours following the ACTH. It is apparent on inspection of Table 3 that there was no correspondence between the percentage decrease of lymphocytes and eosinophils, the per-

centage increase of white blood cells, and the increase in the concentration of 17-hydroxycorticosteroids. If the increase in steroid levels in the peripheral blood may be taken as a measure of adrenocortical activity under the conditions of this experiment, then the percentage change in eosinophils, lymphocytes and white blood cells four hours after ACTH is a very poor quantitative measure of the amount of steroids being secreted by the adrenal.

DISCUSSION

The use of a single intravenous dose of ACTH to test adrenocortical function. The results of this study indicate that even small amounts of ACTH, as little as 0.1 I.U., if given in a single rapid intravenous injection, will produce an increase in the level of 17-hydroxycorticosteroids in the peripheral blood of normal adults. Increasing the dosage through the range of 0.1 I.U. to 30 I.U. does not significantly change the degree to which the blood steroid levels increase at fifteen minutes and one hour after administration of ACTH. It is only at the two-hour and four-hour points that significant differences between dosage effects appear.

Since the significant increments in the level of blood steroids occurred in the range of 0.1 I.U. to 15 I.U. of ACTH, it was concluded that a single intravenous injection of 15 I.U. of ACTH would induce an almost maximal response from the adrenal cortex, and would be a suitable amount to use for testing the upper limits of glandular reactivity to a single, immediate stimulation; whereas 1 I.U. of ACTH, since it induced a consistent response, but a more moderate one, would be suitable as a test agent of glandular reactivity to a milder stimulation. The technique involving the immediate injection of a single dose of ACTH was selected because of its simplicity, with the reservation that the results obtained, although valuable as a measure of the ability of the adrenal gland to respond to stimulation, would be less significant in the assessment of the capacity of the gland to resynthesize and elaborate further hormones once available stores had been exhausted. A more prolonged stimulation, such as an intravenous drip of ACTH over a period of many hours, would test this aspect of glandular function more effectively, and such studies are in progress.

There was such a wide variation from individual to individual in the response to the same amount of ACTH that the normal range was relatively great. This was exemplified by 2 individuals who were studied over the gamut of dosage. One demonstrated maximal rises in steroid concentration at every dosage level of ACTH, whereas the other showed minimal rises. These discrepancies were not explicable in terms of differences in weight, height, age, psychologic status, physical findings, or medical history. A plausible explanation seemed to be a difference in adrenal reaction with-

out adrenal insufficiency, comparable to similar individual variations seen in other areas of physiologic activity.

Consequently, in applying such a test to ill individuals in order to evaluate adrenocortical function, it would be necessary to recognize the wide normal variation. A relatively small increase in the concentration of 17-hydroxycorticosteroids following ACTH given intravenously would not necessarily imply adrenal insufficiency. Slight or moderate impairments in adrenocortical function, when related to disease, could be demonstrated only by a study of a relatively large number of cases, if such small differences are to be distinguished from the normal wide scatter.

On the other hand, the specific individual seemed to react with greater consistency. In spite of variations, when any one subject was studied over a period of months with the same test dose of ACTH, there was a considerable similarity in response for that particular individual. The correlation coefficient with 15 I.U. of ACTH was .70. Therefore, if studies of adrenal function in states of disease could be made, using the individual as his own control, fewer cases would be necessary in order to obtain valid results.

Assay of ACTH in man. All previous assays in man have been dependent upon less precise measures of adrenocortical function such as changes in circulating eosinophils (8), or variations in the excretion of end-products of steroid metabolism found in the urine (8, 9). Using the variation in 17-hydroxycorticosteroids in the peripheral blood two hours after a stimulating dose of ACTH as the measure of adrenocortical response, the log-dose-response curve had a moderate inclination of 11.5 ± 7.61 with a lambda of 0.66 in the range of 1 I.U. to 15 I.U. of ACTH. Although less than ideal, no doubt due to the heterogeneous human population studied as well as the presence of an intact pituitary, this procedure or a modification of it, might have value for the assay of ACTH in man.

Changes in the numbers of eosinophils and lymphocytes as a measure of adrenocortical function in man. Alterations in the number of circulating eosinophils (10) and lymphocytes (11) following a stress has gained popularity as a measure of adrenocortical activity. Recently several reports have appeared which emphasize the unreliability of changes in the numbers of these cells as an index of adrenocortical discharge (12, 13, 14).

Nelson *et al.* (15) reported consistent significant decreases in lymphocytes and eosinophils in association with increases in the level of 17-hydroxycorticosteroids in the blood. However, these results were based upon experimental situations in which the increases in steroid were excessive and unphysiologic.

A part of this study was devoted to the determination of the reliability of the hematologic elements as indicators of adrenocortical function. In-

creases in the blood levels of 17-hydroxycorticosteroids following intravenous ACTH were associated with significant decreases in the total circulting eosinophils, with rare exceptions. However, comparable diminutions in the eosinophils occurred after intravenous placebo injections, and this procedure was accompanied by a progressive drop in the 17-hydroxycorticosteroid titer in several instances. At least in this situation, the significant drop in the total eosinophils could not be ascribed to an increase in adrenocortical function; it may have been related to physiologic processes that were occurring independently of the adrenal. We have seen a comparable asynchrony between steroids and eosinophils in other stressful situations. Occasionally, following electroshock (16), there has been no significant decrease in the total eosinophils, despite a considerable increase in the 17-hydroxycorticosteroids. Adrenalin also induces a remarkable reduction in circulating eosinophils, unassociated with any increase in blood steroid levels (17, 18). For these reasons, changes in the number of eosinophils would seem to be an unreliable indicator of adrenocortical activity. It is true that these cells are sensitive to changes in glandular activity but, as has been indicated, exceptions are not rare. Therefore, unless such changes in the number of eosinophils are associated with concurrent alterations in the level of blood or urinary adrenal steroids, meaningful conclusions cannot be drawn about the state of adrenocortical function.

The lymphocytes proved to be less sensitive than the eosinophils. In 20 per cent of the studies a diminution in the total lymphocytes failed to occur within four hours after ACTH, and in a somewhat smaller percentage of cases no change was evident at the end of either the two-hour or four-hour period. The lymphocytes, too, have proved unreliable at times following electroshock (16), when the level of blood steroids may rise without significant diminution in the lymphocytes.

It seems fair to conclude that the concentration of eosinophils and lymphocytes in the blood of man is influenced by adrenocortical steroids, but other factors affect their concentration sufficiently to make changes in these blood elements dubious indicators of adrenal glandular activity. They should be relied on only when more direct measures of adrenocortical function are not available, with a full realization of their limitations.

Finally, there appeared to be no relationship between the percentage decrease in eosinophils and lymphocytes and the degree to which the level of 17-hydroxycorticosteroids rose. The hematologic elements seem to be unreliable as a *quantitative* measure of adrenocortical activity.

SUMMARY

1. The adrenocortical response of normal adults to the intravenous administration of ACTH given by a single immediate injection in dosages

from 0.1 I.U. to 30 I.U. has been investigated. The resulting change in the concentration of the 17-hydroxycorticosteroids in the peripheral blood has been followed as an indicator of the degree of adrenocortical activity. Concurrent hematologic studies were made in order to determine their reliability as a measure of cortical activity.

2. As little as 0.1 I.U. of ACTH injected intravenously induced a significant increase in the level of 17-hydroxycorticosteroids in the peripheral circulation. A graded adrenal response was demonstrated in the range of ACTH dosage between 1 I.U. and 15 I.U. It is suggested that the increases in the 17-hydroxycorticosteroid level following 1 I.U. and 15 I.U. of ACTH administered intravenously might be used as a test of adrenocortical responsiveness, and could be applied to pathologic states in which some degree of adrenal insufficiency is suspected. The limitations of this technique have been indicated.

3. Any one individual tended to respond in a similar fashion when stressed repeatedly with the same amount of ACTH. The considerable variation between individuals in response to the same amount of ACTH suggests that some people have more labile adrenals than others.

4. The use of this procedure for the biologic assay of ACTH has been investigated.

5. Limitations to the use of changes in the lymphocyte and eosinophil counts in man as a measure of adrenocortical function have been indicated.

Acknowledgments

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CONTINUOUS INTRAVENOUS ACTH INFUSION IN SMALL DOSES AS A PHYSIOLOGIC APPROACH TO TREATMENT

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IT HAS been shown previously that the administration of ACTH to patients in doses much smaller than those commonly employed intravenously gives full therapeutic results without undesirable side reactions (1). The hormone was given as a continuous intravenous infusion, as suggested by Ingle (2), Gordon (3), Renold *et al.* (4), Mandel *et al.* (5) and Jellife *et al.* (6).

In this paper a comparison has been made of the accepted criteria of adrenocortical activity in 21 patients receiving low and high dosages of ACTH. It was found that daily doses of 5 mg. or less by continuous intravenous infusion were therapeutically effective and physiologic in action.

MATERIAL AND METHODS

Twenty-one hospitalized patients (Table 1 and Figs. 1 and 2) suffering from diseases usually responding to ACTH therapy were studied. There were 12 cases of rheumatoid arthritis, 5 of bronchial asthma, 2 of rheumatic fever, 1 of ulcerative colitis and 1 of polyarteritis nodosa.

Cases #1–13 received 1 to 5 mg. of ACTH daily, and Cases #14–21 received 6 to 20 mg. daily. The ACTH (ACTHAR, Armour) was administered by continuous intravenous infusion in from 500 to 1000 ml. of a solution of 5 per cent glucose. The rate of the drip was 12 to 16 drops per minute. If a tendency to clotting was observed at the site of the venepuncture, 3 to 4 drops of a 1 per cent solution of heparin were added to the infusion.

The routine clinical and laboratory examinations for assessing the degree of clinical progress were performed at frequent intervals. All patients were given a salt-free diet, to which sodium chloride (3 Gm. daily) was added in order to ensure a constant daily intake of salt. Twenty-four-hour specimens of urine were collected and kept on ice. The urinary 17-ketosteroids were determined by the method of Drekter *et al.* (7), and also by a micro-method developed by Sulman (8). The daily sodium and potassium excretion in urine was measured with a Barclay flame photometer, and the sugar excretion by the Benedict method. The eosinophil count, in duplicate, was performed by the method of Randolph (9) at 7–8 a.m. daily. In 8 cases the eosinophils were counted

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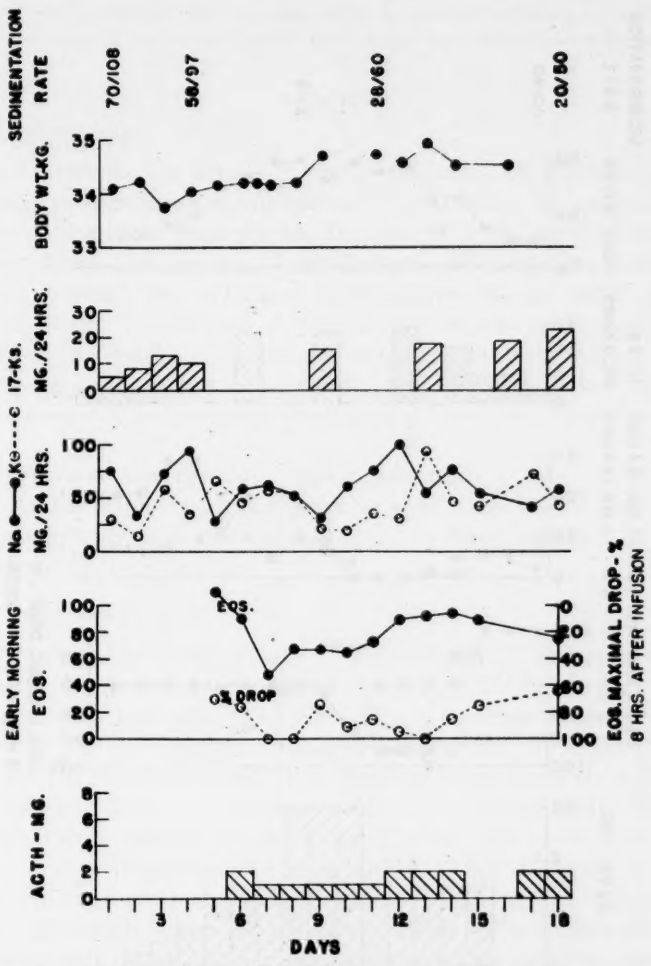

FIG. 1. *Case No. 7, female.* Rheumatoid arthritis treated with low dosage of ACTH (average, 1.5 mg. daily) by intravenous infusion. Good clinical response without unfavorable side reaction; ESR dropped from 70/108 to 20/50; body weight stable; urinary 17-KS within normal limits; Na and K excretion not affected; the eosinophil count showed a slight morning drop, and a 75–100 per cent drop eight hours after the beginning of infusion.

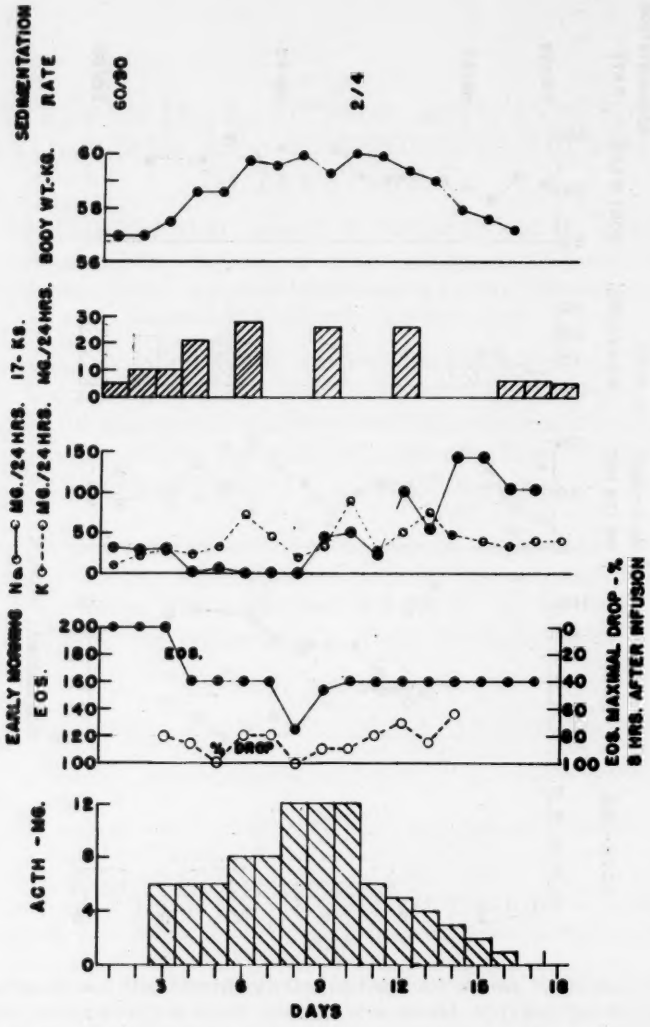

FIG. 2. *Case No. 20, male.* Ulcerative colitis treated with high dosage of ACTH (average, 6.2 mg. daily) by intravenous infusion. Good clinical response, but moon face and flushing also occurred; ESR dropped from 60/90 to 2/4; body weight increased; urinary 17 KS rose to 27.2 mg. per twenty-four hours; there was Na retention; urinary K increased; the eosinophil count showed no morning change, but an 80–100 per cent drop eight hours after the beginning of infusion.

daily, both before, and eight hours after the beginning of the ACTH infusions. Body weight and blood pressure were recorded daily. In patients suffering from rheumatoid arthritis, the range of active and passive movements was determined at frequent intervals.

RESULTS

A. *Clinical response*

Table 1 presents the details of the 21 patients. All patients receiving either small or large doses responded well to treatment. In cases of asthma, the asthmatic attacks disappeared. In cases of rheumatoid arthritis and rheumatic fever, the fever and erythrocyte sedimentation rate (ESR) dropped to normal, the swelling of the joints receded and movements improved markedly. In the case of ulcerative colitis, blood and pus disappeared from the stools (which became normal), tenesmus was eliminated, the rectoscopic picture improved greatly, and the ESR decreased to normal. In the case of polyarteritis nodosa also, there was clinical improvement.

No undesirable side reactions were noted in all 13 patients given low doses of ACTH. However, 6 of 8 patients given large doses showed moon face, flushing, increase in body weight, a rise in blood pressure, or varying combinations of these changes.

B. *17-Ketosteroids in urine*

Table 2 shows the daily urinary 17-ketosteroid (17-KS) excretion. All the patients (except #11) receiving low doses of ACTH (1 to 5 mg.) showed a 24-hour urinary excretion of 17-KS within the normal range.[1] The average maximal excretion was 19.5 mg. per twenty-four hours. On the other hand all the patients (except #19) who received larger doses of ACTH (6 to 20 mg.) showed a 24-hour urinary excretion above normal limits. The average maximal excretion in this group was 27.0 mg. per twenty-four hours. With low dosage treatment, the excretion of 17-KS increased to normal values in the cases in which it was subnormal before ACTH therapy. Only 1 patient (#11) with normal 17-KS excretion before treatment showed an excessive output (up to 36.6 mg. per twenty-four hours) after receiving a daily dose of 5 mg. of ACTH for several days. This patient was extremely emotional.

C. *Circulating eosinophils*

Morning eosinophil counts from fasting patients were lower after ACTH therapy was started than the mean counts before institution of treatment.

[1] In this institution, normal average values of the daily urinary 17-ketosteroid excretion are 12 mg. per twenty-four hours for females and 18 mg. per twenty-four hours for males.

TABLE 1. CLINICAL EFFECT OF INTRAVENOUS INFUSION OF ACTH IN LOW AND HIGH DOSAGE (21 PATIENTS)

	Case No.	Patient	Age (yrs.)	Sex	Disease	Duration of disease	ACTH, av. daily dose (mg.)	ACTH, total dose (mg.)	Duration of treatment (days)	No. of days of treatment until max. relief	ESR (Westergren)		Rise of B.P. during treatment (mm. Hg)	Glycosuria during treatment	Increase in body wt. during treatment (Kg.)	Other side effects
											Before treatment	After treatment				
Low dosage	1	K.S.	50	M	Bronchial asthma	2½ years	2.1	17	8	4	—	—	0	0	0	0
	2	P.M.	16½	F		10 years	1.5	11	7	3	—	—	0	0	0	0
	3	B.M.	39	F		7 years	2.1	17	9	8	35/70	16/30	0	0	0	0
	4	B.A.	44	M		1 year	2.6	34	13	9	20/50	13/34	0	0	0	0
	5	S.H.	38	F		8 months	2	25	13	4	43/75	20/56	0	0	0	0
	6	M.H.	44	F	Rheumatoid arthritis	16 years	2.1	34	16	8	35/65	20/47	0	0	0	0
	7	R.O.	18	F		1 year	1.2	20	16	14	70/108	20/50	0	0	0	0
	8	C.A.	18	M		2 years	2.6	47	18	8	78/117	18/74	0	0	0	0
	9	S.A.	40	M		4 years	1.5	14	9	8	56/85	25/58	0	0	0	0
	10	S.E.	28	F		1 year	1.5	28	21	12	75/112	8/29	0	0	0	0
	11	M.L.	42	F		3 years	3.6	53	15	10	85/105	12/27	0	0	0	0
	12	M.S.	18	F	Rheumatic fever	6 years	1.4	20	14	10	54/80	20/48	0	0	0	0
	13	B.H.	16	M		4 weeks	2	29	14	13	90/130	28/70	0	0	0	0
High dosage	14	G.H.	51	F	Bronchial asthma	7 months	11.3	68	6	3	—	—	20	0	2.6	Flushing; moon face.
	15	L.M.	45	F		1½ years	8	81	10	6	42/65	12/20	20	++	1.5	Started with 15 mg. daily; patient felt worse.
	16	G.M.	57	F		12 years	10	120	12	6	14/30	11/28	0	±	1.2	Moon face. Dose reduced to 4 mg. with good effect.
	17	C.A.	19	M	Rheumatoid arthritis	3 years	20	500	25	10	68/105	10/25	0	0	2.5	Flushing.
	18	A.R.	43	F		6 years	20	420	21	14	42/17	18/35	50	0	0	Moon face.
	19	K.R.	42	F		2 years	15	225	15	15	40/17	15/30	0	0	1.5	Moon face.
	20	T.M.	37	M	Ulcerative colitis	3 years	6.2	87	14	3	60/90	2/4	20	0	3	Moon face; flushing.
	21	H.E.	43	M	Polyarteritis nodosa	½ year	11	139	13	10	28/85	0/1	0	0	0	0

TABLE 2. 17-KETOSTEROID EXCRETION IN URINE AND EARLY MORNING EOSINOPHIL COUNT DURING INTRAVENOUS INFUSION OF ACTH IN LOW AND HIGH DOSAGE (21 PATIENTS)

	Case No.	Age	Sex	Disease	ACTH treatment				17-Ketosteroids in urine (mg./24 hrs.)				Eosinophils (early morning count)			
					Duration (days)	Total dose (mg.)	Avge. daily dose (mg.)	Max. daily dose (mg.)	Mean before treatment (mg./24 hrs.)	After first infusion (mg./24 hrs.)	Max. excretion (mg./24 hrs.)	Mean during treatment (mg./24 hrs.)	Mean before treatment (per cu. mm.)	Lowest count (per cu. mm.)	Mean during treatment (per cu. mm.)	Drop %
Low dosage	1	50	M	Bronchial asthma	8	17	2.1	3	6.5	16.4	16.4	14.5	310	66	131	58
	2	16½	F		7	11	1.5	3	17	18.9	18.9	16.3	180	165	170	0
	3	39	F		9	17	2.1	3	6.5	9.9	14	11.5	322	163	204	37
	4	44	M		13	34	2.6	4	12	14.3	16.3	13	168	55	156	7
	5	38	F		13	25	2	3	16	15.9	21	17.7	183	22	152	17
	6	44	F	Rheumatoid arthritis	16	34	2.1	4	4	18.2	18.2	15.2	135	27	98	28
	7	18	F		16	20	1.2	2	7.7	14.7	21	16.9	187	44	150	20
	8	18	M		18	47	2.6	3	9	18	20.9	19.8	151	121	390	258% increase
	9	40	M		9	14	1.5	4	8.1	16.9	18.7	17.1	175	100	208	118% increase
	10	28	F		21	28	1.5	3	3	10.5	18.7	12.7	218	11	125	41
	11	42	F		15	53	3.6	5	11.6	22	36.6	28.6*	302	22	74	76
	12	18	F	Rheumatic fever	14	20	1.4	3	6	12	12	9.2	180	66	132	89
	13	16	M		14	29	2	3	5.5	8.4	21	12.4	1,144	407	936	19
High dosage	14	51	F		6	68	11.3	15	16	20	30	25	3,734	66	255	93
	15	45	F	Bronchial asthma	10	81	8	15	1.5	6.3	21	12.8	473	121	191	60
	16	57	F		12	120	10	10	5.4	8	29.3	15.3	550	253	418	24
	17	19	M		25	500	20	20	16	25.5	31	23	241	11	45	72
	18	43	F	Rheumatoid arthritis	21	420	20	20	8.4	11.9	25.5	16.4	350	22	36	87
	19	42	F		15	225	15	15	8	14	17	13	250	27	35	86
	20	37	M	Ulcerative colitis	14	87	6.2	10	8	9.6	27.2	23.5	174	95	150	12
	21	43	M	Polyarteritis nodosa	13	139	11	15	11.2	28	33.6	25.7	85	22	46	54

* Extremely emotional patient.

TABLE 3. PERCENTILE FALL IN EOSINOPHILS AT END OF EIGHT-HOUR ACTH INFUSION (8 PATIENTS)

	Case No.	Disease	1st Day		2nd Day		3rd Day		4th Day		5th Day		6th Day		7th Day	
			ACTH (mg.)	Drop %	ACTH (mg.)	Drop %	ACTH (mg.)	Drop %	ACTH (mg.)	Drop %	ACTH (mg.)	Drop %	ACTH (mg.)	Drop %	ACTH (mg.)	Drop %
Low dosage	5	Rheumatoid arthritis	3	100	2	85	2	18	2	81	2	72	2	92	2	75
	7	Rheumatoid arthritis	3	70	2	76	3	100	3	100	2	75	3	91	3	85
	8	Rheumatoid arthritis	3	76	2	63	2	57	2	73	2	49	3	86	3	82
	13	Rheumatic fever	3	90	2	93	2	86	2	100	2	100	2	40	1	40
High dosage	15	Bronchial asthma	15	80	10	65	4	79	4	67	4	80	4	69	4	80
	16	Bronchial asthma	10	24	10	92	10	92	10	95	10	100	10	100	10	100
	20	Ulcerative colitis	6	80	6	85	6	100	8	80	8	80	12	100	12	90
	21	Polyarteritis nodosa	15	100	15	100	15	100	15	100	20	100	20	100	15	98

However, the percentile drop was variable from patient to patient, and inconsistent within the same patient (Table 2). The amount of decrease was not always correlated with the size of the dose administered on the previous day. In patients who received small doses, the fall varied between 7 and 76 per cent, and in patients receiving large doses, the mean drop was between 12 and 93 per cent. In most of the cases there was an initial morning eosinophil drop during the first few days of treatment. Later in the course of therapy the morning eosinophil count was increased.

In 4 patients (#5, 7, 8 and 13) receiving small doses of ACTH, eosinophil counts were made daily, both directly before, and eight hours after the start of the ACTH infusions. In these, the drop varied between 40 and 100 per cent (Table 3). In 4 patients receiving large doses of ACTH (#15, 16, 20 and 21) the drop eight hours after beginning the infusion was between 80 and 100 per cent, after the first day of treatment.

D. *Daily electrolyte excretion in urine*

Table 4 shows the daily excretion of sodium and potassium. Patients #1–13, who received small doses of ACTH, showed no sodium retention or increased potassium excretion in the urine. Patients #14–21, receiving large doses of ACTH, showed retention of sodium during the first few days of treatment, followed later by a rise of the sodium excretion (with the exception of Case #17). At the same time there was a comparatively greater output of potassium in the urine of these patients.

E. *Glycosuria*

Glycosuria was not found in any patient receiving small doses of ACTH. There were 2 cases of transient glycosuria in the group given large doses (Cases #15 and 16).

DISCUSSION

The clinical response of all patients given 1 to 5 mg. of ACTH daily was good, and compared favorably with the response in those who received 6 to 20 mg. daily. Undesirable side effects, however, described by other authors (10, 11, 12) were noted in 6 of 8 cases treated with large doses. No such effects were encountered in the 13 cases of the low-dosage group. The side effects were absent also in 15 other cases treated with the low doses and described previously (1).

The daily excretion of 17-KS in the urine in those patients receiving small doses of ACTH, if subnormal before treatment, rose to within normal limits; if normal before treatment, it rose, but was still within the normal range (average maximum 19.5 mg. per twenty-four hours). On the other hand, patients who received large doses of ACTH showed a rise in 17-KS excretion far above normal limits (average maximum, 27.0 mg. per twenty-

TABLE 4. URINARY SODIUM AND POTASSIUM EXCRETION (MG./24 HRS.) DURING FIRST FIVE DAYS OF TREATMENT WITH ACTH IN LOW AND HIGH DOSAGE BY INTRAVENOUS INFUSION (21 PATIENTS)

Case No.	ACTH, avge. daily dose		Mean before treatment	Day of treatment				
				1st	2nd	3rd	4th	5th
			Low dosage					
1	2.1	Na	45	35	31	44	24	100
		K	15	27	39	39	12.5	39
2	1.5	Na	70	40	63	77	64	86
		K	40	71	35	82	8.5	29
3	2.1	Na	75	74	60	65	42	72
		K	46	69	55	54	50	48
4	2.6	Na	69	33	27	23	22	36
		K	31	65	57	36	41	27
5	2	Na	96	31	100	18	144	85
		K	49	81	75	11	79	55
6	2.1	Na	81	44	22	24	30	27
		K	24	27	35	43	27	13
7	1.2	Na	75	20	60	67	63	33
		K	28	38	65	51	25	19
8	2.6	Na	67	33	98	97	95	72
		K	56	60	37	61	55	63
9	1.5	Na	136	50	70	26	62	150
		K	25	25	75	26	75	74
10	1.5	Na	100	100	102	26	28	52
		K	40	25	26	50	25	52
11	3.6	Na	39	33	10	86	140	60
		K	22	34	13	27	60	51
12	1.4	Na	56	125	82	12	100	60
		K	21	30	25	25	52	50
13	2	Na	69	43	30	56	90	70
		K	49	46	32	36	21	32

TABLE 4—(*continued*)

Case No.	ACTH, avge. daily dose		Mean before treatment	Day of treatment				
				1st	2nd	3rd	4th	5th
				High dosage				
14	11.3	Na	45	0	12.5	3	0	0
		K	16	6.5	12.5	25	50	50
15	8	Na	48	35	12	6	15	7
		K	25	30	28	88	38	29
16	10	Na	35	24	5	10	0	46
		K	20	24	29	23	58	9
17	20	Na	37	18	8	25	30	60
		K	50	36	35	35	23	12
18	20	Na	43	33	10	11	50	62
		K	44	80	54	54	62	64
19	15	Na	57	30	20	4	3	0
		K	55	18	25	30	32	27
20	6.2	Na	29	26	0	8	0	0
		K	20	25	24	34	69	48
21	11	Na	58	45	3	1	0	64
		K	83	56	25	51	63	26

four hours). This elevated response to large doses has been reported also by Renold *et al.* (10), who gave 20 mg. of ACTH intravenously over an eight-hour period. Most of their 20 patients treated with this dose had a maximal rise in 17-KS up to 35 mg. per twenty-four hours. The elevation and maintenance of 17-KS excretion within the normal range in 12 patients given small doses of ACTH indicated that the adrenal cortex was efficiently stimulated, but not overstimulated by such doses.

Urinary 17-KS excretion proved in this series to be the most reliable index of adrenocortical stimulation, since it ran almost parallel with the dosage of ACTH administered.

The eosinophil count, on the other hand, was not found to be a reliable index of ACTH activity. The early morning eosinophil count decreased in some cases and remained at the control level in others, after institution of therapy. With low dosage, there was a drop in the count eight hours after

the beginning of the infusion. This drop was not constant from day to day in the same person after the same dose of ACTH over the same period of time. With larger doses the counts often fell to zero. This conforms with the observation of Renold *et al.* (10). These data have emphasized that a positive eosinophil response to ACTH merely indicates the presence of functioning adrenal cortex but is not a quantitative measure of adrenocortical activity. No constant and proportional relationship was found between the degree of eosinophil fall and the quantity of ACTH administered.

The lack of sodium retention and the excess of potassium excretion in the patients given small quantities of ACTH indicated that at this dosage level the medication did not interfere with the mineral metabolism, *i.e.*, 1 to 5 mg. of ACTH, though clinically effective, did not cause sufficient discharge of mineral corticoids to disturb normal electrolyte metabolism. In these cases there was no evidence of a marked rise in blood pressure or body weight, "moon face," or flushing, as often noted in patients receiving larger doses of ACTH. Similarly, overproduction of glucocorticoids, as manifested by glycosuria or hyperglycemia, was not observed.

The good therapeutic effects of small doses of ACTH, the lack of rise above normal limits of the 24-hour 17-KS excretion, and the lack of any observable disturbances in the electrolyte metabolism, showed a dissociation of the therapeutic from the pharmacologic effect of these doses. These observations indicated that daily doses of from 1 to 5 mg. of ACTH were physiologic in their action on the adrenal cortex. At higher dosage levels the hormone exerted a pharmacologic effect, overstimulating the adrenal cortex, as manifested by the development of undesirable side reactions. Thus, large doses are to be avoided in those patients who suffer simultaneously from such conditions as hypertension, diabetes, or heart disease.

SUMMARY

1. Twenty-one patients with rheumatoid arthritis, rheumatic fever, bronchial asthma, polyarteritis nodosa or ulcerative colitis were treated with daily ACTH infusions over a period of eight to twelve hours. Thirteen of these patients were given small daily intravenous doses of from 1 to 5 mg. of ACTH. The other 8 patients were treated with daily intravenous doses from of 6 to 20 mg. of ACTH. The therapeutic results were equally good in all cases.

2. Patients receiving small doses of ACTH showed a 24-hour excretion of 17-ketosteroids which was within normal limits but did not exceed normal values (maximum 21 mg.). Patients receiving larger doses of ACTH showed an excretion which exceeded normal limits (up to 33.6 mg.). With small doses of ACTH, the 24-hour 17-ketosteroid excretion was found to be the most reliable index of adrenocortical activity.

3. Patients receiving small doses of ACTH showed a moderate drop in eosinophil count eight hours after the infusion was started. The fall in the early morning eosinophil count was not constant during therapy.

4. In patients receiving small doses of ACTH, no disturbance of the electrolyte or carbohydrate metabolism was observed.

5. It is suggested that continuous intravenous treatment with small doses of ACTH (from 1 to 5 mg. per day) represents a physiologic method of treatment, giving optimal results.

6. The low-dosage schedule may be of particular value for those patients who need ACTH therapy and who suffer simultaneously from such conditions as hypertension, diabetes, or heart disease. Large doses of ACTH may often aggravate these latter conditions and cause dangerous side reactions.

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ANDROGENIC ACTIVITY PER MILLIGRAM OF COLORIMETRICALLY MEASURED KETOSTEROIDS IN URINE: AN INDEX OF THE RESPECTIVE CONTRIBUTIONS FROM TESTICULAR AND EXTRA-TESTICULAR SOURCES*

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THE theses of this communication are that the number of international units (I.U.) of androgens per milligram of colorimetrically measured urinary ketosteroids a) reflects the relative proportions of urinary ketosteroids[1] derived from testicular and extra-testicular sources and b) can be used as an index of the respective degrees of function of testicular versus extra-testicular sources.

Urinary androgenic activity in the normal human adult depends chiefly upon androsterone (Fig. 3), the bulk of which is dervied from testicular secretions in normal men (2, 3, 4). Androsterone is present in large quantities in human urine (Fig. 2) and has a much higher androgenic activity than that of any compound originating primarily from adrenocortical or other extra-testicular sources (3).

In contrast to androgenic values, colorimetric assays of urinary ketosteroids are a "blanket" measurement of the total quantity of ketosteroids, without regard to their androgenic activity. Thus an index afforded by the androgenic activity per milligram of colorimetrically determined urinary ketosteroids represents a balance that in males is struck between the proportions of a) highly androgenic metabolites derived largely from testicular secretions (chiefly androsterone) and b) ketosteroids with less androgenic activity, derived chiefly from extra-testicular sources.

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[1] The term "ketosteroids" is used in preference to the more common designation of "17-ketosteroids." Use of this term is based on the fact that the methods employed recover compounds that include 3- and 20-, as well as 17-, ketosteroids (1). Alpha-ketosteroids are the hydroxy compounds not precipitated upon the addition of digitonin to the neutral ketonic fraction. Beta-ketosteroids include hydroxy compounds that are precipitated upon treatment with digitonin.

In general, the values for this index in males are low before puberty and increase at the time of sexual maturation to reach a peak in the second and third decades of life. The index declines appreciably with advancing age, and even more markedly upon castration. Values appear to be greatly elevated in subjects whose testicular secretions are supranormal as a result of interstitial-cell tumors of the testis.

In females the average number of I.U. of androgens per mg. of colorimetrically measured urinary ketosteroids is also low prior to maturity and rises markedly at the time of sexual maturation. The status in females differs from that in males, however, in that a) the index does not reach as high a level in young adults, and b) the reduction upon gonadectomy is not sufficient to reach levels observed in eunuchs. Available data for subjects with androgen-secreting tumors of the ovaries are limited, but suggest that the index is not elevated to the extent observed in subjects with interstitial-cell tumors of the testes.

The theses of the present communication are at variance with the almost universally prevailing viewpoint (5–11) that colorimetric assay of ketosteroids reliably gauges the androgenic activity of the mixture of urinary ketosteroids, whatever the clinical state of the subject. Callow, Callow and Emmens (6), in an analysis of a variety of endocrine states that included normal persons of both sexes and various ages, eunuchism, eunuchoidism and adrenocortical hyperplasia, state that "the content of biologically active material is proportional to the content of 17-ketosteroid compounds." These authors reported a coefficient of correlation of 0.745. Zarrow and associates (11) reported a similar coefficient of correlation of 0.73, and Holtorff and Koch (7) one of 0.42. Dorfman, on the basis of his own work and an excellent review of the literature pertaining to androgenic compounds (10), concluded that "a reasonable parallelism exists between the quantity of androgenic material (biologic assay) and the amount of 17-ketosteroids (chemical assay)."

As shown later, however, the actual data obtained by some of the aforementioned investigators and by others, including this author, support the theses of the present communication.

MATERIALS AND METHODS

In an attempt to increase the reliability and comparability of data for subjects with a wide range of age, the following analysis is based chiefly upon, and wherever possible is restricted to, data which meet the following requirements:

1) That both androgenic and colorimetric assays be carried out on the same subjects.

2) That subjects be normal rather than ill.

3) That techniques be used which permit comparison with work from other laboratories.

4) That data be provided by the same laboratory for a large number of subjects covering a wide range of age. This avoids errors introduced when comparisons are based on data supplied by many investigators who use somewhat different techniques.

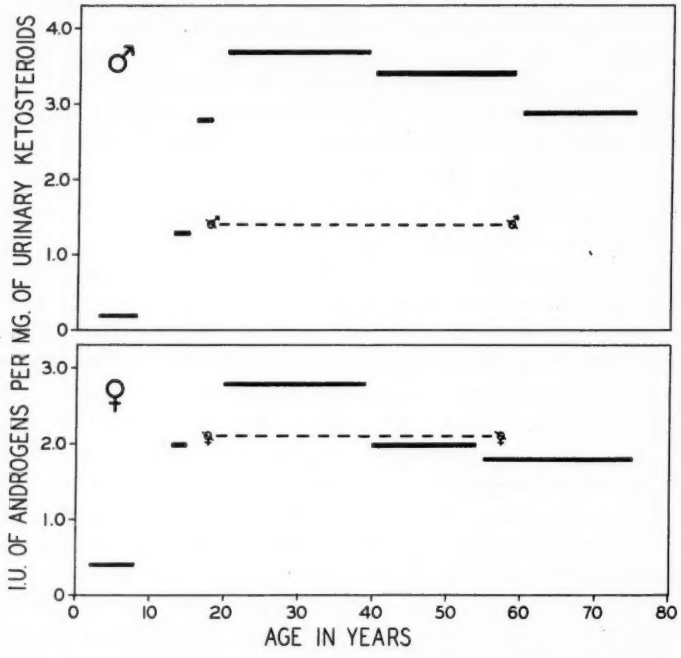

FIG. 1. The index (I.U. of androgenic activity per mg. of colorimetrically determined ketosteroids) increases upon sexual maturation and decreases afterwards upon aging. The direction of change is similar in both sexes, but absolute values are lower in women than in men.

Because of the aforementioned restrictions, the following analysis is based chiefly upon work from three laboratories, those of Hamburger,[2] the Callows and the writer. Values obtained by other investigators are listed in Tables 1 to 3 as supplementary data.

The writer's data for urinary androgens and ketosteroids are given in detail elsewhere (14, 15), along with a description of the subjects and procedures utilized in assays.

[2] Values for studies by Hamburger (12) and associates (13) were calculated from graphic material.

RESULTS

General statement of status in males (Table 1 and Figure 1)

The average number of I.U. of androgens per mg. of urinary ketosteroids (referred to henceforth as the index) in young men 20 to 39 years of age was 3.7. Lower values were found in states in which testicular secretions are considered to be less than in young men, namely a) boys 3 to 8 years old (index, 0.2); b) boys 13 to 15 years old (index 1.3); c) boys 16 to 18 years old (index 2.9); d) eunuchs (index 1.4); e) old men 60 to 75 years old (index 2.9); and f) a subject with primary pituitary insufficiency (index 0.3).

High indices would be expected in conditions in which testicular secretions are selectively augmented, such as occurs in some subjects with interstitial-cell tumors of the testes. The 2 cases which have been reported in detail (16–18) would appear to have exceptionally high values.

In normal men the peak values for the index (I.U. of androgens per mg. of colorimetrically measured urinary ketosteroids) are observed in young adults

Table 1 shows an excellent agreement of data from three laboratories in studies of 46 normal young men in the third and fourth decades of life (indices of 3.5, 3.9 and 3.9, with a mean of 3.7).

Indices lower than the mean in young men may be found if testicular contributions to urinary ketosteroids are less than in young men

1. *Immature boys (Table 1).*[3] The index was only 0.2 in 8 boys 3 to 8 years of age. It rose to 1.3 in 19 boys 13 to 15 years of age and to 2.9 in 4 boys 16 to 18 years old. The latter value was only 22 per cent less than in men at 20 to 39 years of age.

2. *Castrate and eunuchoid males (Table 1).* In 21 eunuchs (whose testicular secretions are eliminated completely, whereas adrenocortical and perhaps other sources of precursors of urinary metabolites continue to function—supranormally (6) in some instances), the mean index was 1.4, only 38 per cent of that in normal young men.

Eunuchoidism includes a variety of dissimilar states. In some cases subnormal titers of gonadotropins and congenital defects point to abnormalities in the anterior lobe of the pituitary gland (21) and the central nervous system (26). In such subjects the index may be low, with the values in some cases approaching the 0.3 observed in an individual with primary pituitary insufficiency (27). In types of eunuchoidism in which testicular

[3] These data do not include the first few days of life when there is a transient period of testicular function as a result of prenatal stimulation by placental and maternal hormones (10).

TABLE 1. I.U. OF ANDROGENS PER MG. OF URINARY KETOSTEROIDS IN NORMAL MALES AT DIFFERENT AGES AND IN MALES WITH VARIOUS STATES OF TESTICULAR INSUFFICIENCY

Subjects		Total KS		Androgens		I.U. of androgens per mg. of KS	Reference
Status	Age in yrs.	No. of subjects	mg./24 hrs.	No. of subjects	I.U./24 hrs.		
Immature children	3–8	7	0.8	5	1.0	1.2	(12, 13)
(male)	3–8			3	1.0		(19)
	3–8	28	3.8				(20)
Average	*3–8*	*35*	*4.6*	*8*	*1.0*	*0.2**	
Adolescent males	13–15	2	3.7				(12)
	13–15	10	10.6			1.6	(5)
	13–15	7	13.7				(20)
	13–15			30	18.0		(13)
	13–15			62	11.0		(19)
Average	*13–15*	*19*	*11.0*	*92*	*13.3*	*1.3**	
Average	*16–18*	*4*	*7.6*	*17*	*22.1*	*2.9**	(12, 13)
Young men	21–39	17	10.6	13	40.9	3.9	(14, 15)
	20–39	26	15.1	26	52.0	3.5	(12, 13)
	20–39	22	13.0				
	—	7	8.0†	7	31.4†	3.9*†	(6)
Average	*20–39*	*72*	*12.5*	*46*	*45.7*	*3.7*	
Middle-aged men	40–59	20	6.9	13	24.1	3.5	(14, 15)
	40–59	26	11.9	16	40.0	3.4	(12, 13)
Average	*40–59*	*46*	*9.7*	*29*	*32.9*	*3.4**	
Old men	60–75	10	4.2	4	7.4	1.8	(14, 15)
	60–75	21	5.3	14	16.1	3.0	(12, 13)
Average	*60–75*	*31*	*4.9*	*18*	*14.2*	*2.9**	
Eunuchs	19–46	8	5.9†	8	8.4†	1.4†	(6)
	28–52	10	5.6				(12)
	47–66	3	5.2				(21)
	21, 56			2	4.0		(22)
	39–56			4	7.7		(23)
	26–57			14	8.1		(24)
Eunuchoids	—	2	14.1				(25)
	42, 56			4	12.3		(25)
	20–35	12	4.5				(21)
	20–36			8	18.5		(22)
	18–42			7	18.7		(23)
Average	*18–56*	*14*	*5.6*	*19*	*17.6*	*3.1**	

* Androgenic values and colorimetric KS values do not necessarily apply to the same individuals.
† Values calculated per liter of urine.

function is disturbed to various degrees, the range of decrease in testicular secretions varies from much to practically nil. The mean index in the 14 cases listed in Table 1 is 3.1, but this value is of questionable significance since the colorimetric and androgenic assays were not carried out on the same subjects.

3. *Middle-aged and old men* (*Table 1*). The mean index was 3.4 in 46 men 40 to 59 years old, and 2.9 in 18 old men in the seventh and eighth decades of life. The average values in old men are 22 per cent lower than in young men. Because of the lapse of time since Dr. Callow carried out his studies,

he could not supply the writer with values in individual subjects for statistical treatment of the age differential of 22 per cent. Statistical examination was restricted, therefore, to data from the writer's laboratory in which, despite the limited amount of data, the difference between the index in 14 men 21 to 39 years old and 4 men 60 to 75 years old is significant at the 1 per cent level (15).

Indices lower than the mean in young men may be found if extra-testicular contributions to urinary ketosteroids are increased relative to those of testicular contributions

Theoretically the index might be low if contributions from extra-testicular sources were exceptionally large and if their metabolites did not consist in great part of a moderately active androgen such as dehydroisoandrosterone. No studies of males with adrenal cancer, in which both androgenic and colorimetric assays were made in the same patients, have come to the

TABLE 2. I.U. OF ANDROGENS PER MG. OF URINARY KETOSTEROIDS IN NORMAL FEMALES AT DIFFERENT AGES AND IN FEMALES AFTER BILATERAL OÖPHORECTOMY OR THE ONSET OF ADRENAL CANCER

Subjects		Total KS		Androgens		I.U. of androgens per mg. of KS	Reference
Status	Age in yrs.	No. of subjects	mg./24 hrs.	No. of subjects	I.U./24 hrs.		
Immature children	2–8	5	0.8				(12)
(female)	3–8	34	3.4				(20)
	3–8			11	@1.6		(10)
Average	*2–8*	*39*	*3.0*	*11*	*@1.6*	*0.5**	
Adolescent (females)	13–15	5	5.6	2	17.0	3.1	(12)
	13–15	5	10.8				(5)
	13–15	5	9.8				(20)
	13–15			6	17.5		(10)
Average	*13–15*	*15*	*8.7*	*8*	*17.3*	*2.0**	
Young women	20–39	35	8.3	35	22.9	2.8*	(12, 13)
	—	[—	8.9†	6	32.1†	3.6*†	(6)
Average				*41*	*23.4*		
Middle-aged women	40–54	26	5.7	10	11.6	2.0*	(12, 13)
Old women	55–60	2	5.0	3	4.0	0.8*	(12, 13)
	60–75	25	3.3				(12)
	65, 72			2	<9, 11		(28)
Average	*55–75*	*27*	*3.4*	*5*	*6.2*	*1.8**	
Ovariectomized	19–56	11	8.3†	15	17.1†	2.1*†	(25)
women	27–45	5	9.0				(21)
Adrenal cancer	6	1	850.6	1	2220.0	2.6	(29)
	8	1	83.0	1	350.0	4.2	(30)
	25	1	64.0	1	145.0	2.3	(29)
Average	*6–25*	*3*	*332.3*	*3*	*898.3*	*2.7*	

* Androgenic values and colorimetric KS values do not necessarily apply to the same individual.
† Values calculated per liter of urine.

writer's attention. In 3 females with adrenal cancer and high titers of ketosteroids the mean index was similar to that in young women (Table 2).

Indices higher than the mean in young men may be found if testicular contributions to urinary ketosteroids are increased relative to those of extratesticular contributions

Extraordinarily great secretory activity by the testis, resulting in supranormal quantities of androsterone in blood and urine, both in absolute terms as well as relative to other ketosteroids, has been reported in 2 subjects with interstitial-cell tumors of the testis (16–18). In the 41-year-old subject studied by Venning, Hoffman and Browne (16, 17) 600 to 1,000 mg. of urinary ketosteroids were excreted daily. From 483 mg. of keto-

TABLE 3. I.U. OF ANDROGENS PER MG. OF URINARY KETOSTEROIDS IN CERTAIN ENDOCRINE STATES

Subjects			Total KS		Androgens		I.U. of androgens per mg. of KS	Reference
Status	Sex	Age in yrs.	No. of subjects	mg./24 hrs.	No. of subjects	I.U./24 hrs.		
Primary pituitary insufficiency	M	42	1	2.6	1	0.8	0.3	(27)
	M	14–55	7	<0.5–1.8				(21)
	M	30	1	1.6				(36)
	M	18	1	0.6				(37)
	F	20–45	7	<0.5				(21)
Hyperthyroidism	M	38, 40	2	8.6	2	59, >44	5+	(25)
	M	32, 51	6	8.8	6	6.4	0.73	(38)
Average	*M*	*32–51*	*8*	*8.8*	*8*	*17.5*	*2+*	
	F	20, 40	2	3.6	2	2.0	0.56	(25)
	F	20	2	7.7	2	2.0	0.26	(38)
Average	*F*	*20–40*	*4*	*5.6*	*4*	*2.0*	*0.4*	
Addison's disease	M	—	1	2.0†	1	1.0†	0.5†	(6)
	M	—	1	2.9†	1	12.0†	4.1†	(6)
	M	—	1	3.4†	1	17.0†	5.0†	(6)
Average	*M*	—	*3*	*2.8†*	*3*	*10.0†*	*3.6†*	
	M	33–58	3	2.7				(21)
	M	21–34	9	8.5				(10)
	F	—	4	4.5†	4	11.1†	2.47†	(6)
	F	23–64	5	<0.5				(21)
	F	30, 38	2	3.1				(10)

† Values calculated per liter of urine.

steroids, these investigators isolated 136 mg. of androsterone, 31 mg. of etiocholanone, 19.1 mg. of androstenone, 3.1 mg. of isoandrosterone, but no dehydroisoandrosterone. The I.U. per mg. of urinary ketosteroids was obviously high, since androsterone has by far the highest androgenic activity of all urinary steroids. The number of I.U. of androgens per mg. of urinary ketosteroids cannot be computed from the data of Venning and co-workers, but a conservative estimate[4] would be in the neighborhood of 6.

[4] The author is indebted to Dr. Thomas Gallagher for this rough appraisal.

In the 6-year-old boy studied by Cook and associates (18) no index can be calculated, but chromatographic analysis of urinary ketosteroids again revealed an extraordinarily large fraction in the region attributed to 3-alpha-hydroxy 17-ketosteroids and identified with androsterone.

Indices in miscellaneous conditions: anorexia, inanition, primary pituitary insufficiency, and hyperthyroidism

Anorexia and inanition are known to reduce testicular secretions (31–34) and urinary ketosteroids (21, 35).

A low index of 0.3 was reported for a man with primary pituitary insufficiency (Table 3). If studies of further cases confirm this finding of an index less than in eunuchs, it would suggest that values even as low as the 1.4 in eunuchs can be further decreased by failure of the anterior lobe of the pituitary gland to stimulate the production of androgenic precursors by extra-testicular sources.

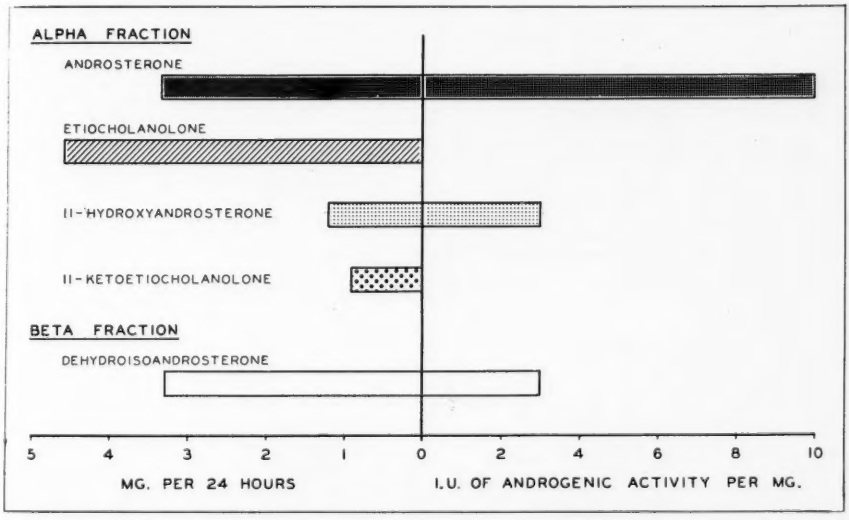

FIG. 2. The most important of the ketosteroids found in urine are compared with regard to androgenicity and the average quantities per twenty-four hours isolated from urines of 10 normal young men 21 to 35 years of age. Isolation data for the alpha-ketosteroids were supplied by Dr. Thomas Gallagher from studies carried out at Sloan-Kettering Institute (4, 40). Data for dehydroisoandrosterone are based on studies by Ronzoni (41) of only 4 men of unstated age.

Values for the androgenic activity of these compounds are those listed by Dorfman (10) and supplemented in a personal communication. Corrections for chromogenicity were furnished by Dr. Seymour Lieberman.

The foregoing graph is based on the following tabular data:

Ketosteroids in urine	Fraction	Colorimetric values per 24 hrs.			Correction for chromogenicity	Androgenic activity		Presumed source of precursors	Per cent of urinary androgenic activity accounted for in	
		Observed								
		mg.	% of total extract	% of alpha fraction		I.U. per mg.	Type of assay		Alpha plus beta fractions	Alpha fraction
Androsterone	Alpha	3.34	25	33	1.00	10.0	Capon	Chiefly testis but also adrenal (orelsewhere)	71	90
Etiocholanolone	Alpha	4.60	34	46	1.12	<0.2	Chick	Chiefly testis but also adrenal (orelsewhere)	—	—
11-Hydroxy-androsterone	Alpha	1.21	9	12	0.62	3.0	Chick	Adrenal	7	10
11-Ketoetio-cholanolone	Alpha	0.92	7	9	1.17	If active, <0.5	Chick	Adrenal	—	—
Dehydroiso-androsterone	Beta	3.3	25	—	1.00	3.0	Capon	Adrenal	22	—

In hyperthyroidism the index was also low in all cases (Table 3) except in the 2 men studied by Callow and associates (25).

Chemical basis for the thesis that the index reflects the proportions of urinary ketosteroids contributed by testicular vs. extra-testicular sources

Androsterone is the most highly androgenic compound in urine (3, 39) and is chiefly responsible for the androgenic activity of urines in normal young men (3, 4), as illustrated in Figures 2 and 3.

Androsterone and its practically nonandrogenic isomer, etiocholanolone, comprise almost all of the urinary ketosteroids derived from testicular precursors (4). Evidence that these compounds originate in testicular secretions was obtained when testicular secretions were enhanced by treatment of patients with chorionic gonadotropins (42). Previous studies had

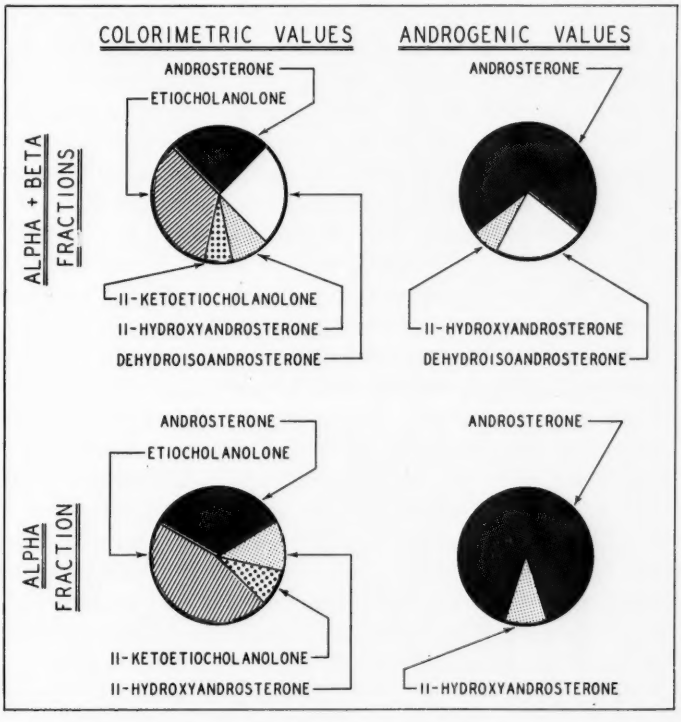

FIG. 3. Androsterone, a compound which is derived chiefly from testicular secretions (4), is responsible in young men for most of the androgenic activity of the urine, especially for the activity of the alpha fraction. Colorimetric values are influenced by metabolites of adrenocortical secretions to a greater extent than are androgenic values. This graph is based on data tabulated in the legend of Figure 2.

shown that the administration of testosterone, a substance which has been isolated from testes (43, 44), results in increased quantities of urinary androgens (45, 46), of androsterone (4, 47, 48) and of its isomers etiocholanolone (4, 47, 49) and isoandrosterone (50), but not of urinary substances derived from adrenal secretions (51).

Androsterone is also derived from extra-testicular secretions, since it is present in the urine of eunuchs (4, 52) and women (2, 4) and has been shown to be a metabolite of dehydroisoandrosterone (53), Δ^4-androstenedione (54), and 17-alpha-hydroxyprogesterone (55).

It is clear, however, that the quantities of androsterone and its precursors from extra-testicular sources are not sufficient to exert any great androgenic effect. The androgens in men who have been orchiectomized prior to puberty do not stimulate growth of secondary sexual characters such as axillary hair (56) and beard, or produce common baldness (57). A degree of response by some of these secondary sex characters can be induced by small amounts of androgens if treatment is prolonged (58); hence the lack of such response in eunuchs is a physiologic demonstration that androsterone and its precursors from extra-testicular sources are insufficient to produce, even in rather small degree, the ordinary androgenic responses in secondary sex characters. This lack of androgenic effect from androgens derived from extra-testicular sources is in harmony with the low index of 1.4 in eunuchs in comparison with the index of 3.7–3.4 in normal men of comparable age.

Androgenic ketosteroids derived from extra-testicular sources such as the adrenal cortices are present in urine in a somewhat larger quantity than is androsterone, but they are much less active per mg. (Fig. 2). Consideration need be given only to two urinary ketosteroids, dehydroisoandrosterone and 11-hydroxyandrosterone, which originate in precursors produced by the adrenal cortices. They are present normally (3, 4) in urine (Figs. 2 and 3), but would seem to account for less than one third of the total urinary androgenic activity of normal young men (Fig. 3).

The origin of dehydroisoandrosterone from adrenocortical secretions is attested by its presence in supranormal quantitites in patients with adrenal cancer (29, 59), in subjects treated with adrenocorticotropins (51), and by the reduced amounts observed in persons with poor adrenocortical function as a result of Addison's disease (59). Isolation procedures, and the Pettenkofer chromogenic test for dehydroisoandrosterone and its hydrolytic artefacts (60) have demonstrated that urinary dehydroisoandrosterone is not derived from testicular secretions, as shown by studies a) comparing men, women and eunuchs (3); b) of patients with enhanced testicular secretions as a result of interstitial-cell tumors (16) or treatment

with chorionic gonadotropins (51); and c) of subjects given testosterone (47, 48, 51).

In brief, colorimetric assays are a measure of all ketosteroids regardless of their androgenic activity, whereas biologic assays measure only androgenic activity, which is due chiefly to androsterone (Fig. 2), a derivative of testicular secretions. Although androsterone is also derived from the extratesticular sources (as detailed previously), the number of I.U. of androgens per mg. of colorimetrically measured urinary ketosteroids in males seems to reflect in an over-all fashion, the relative contributions of testicular *vs.* extratesticular sources.

I.U. of androgens per mg. of colorimetrically measured urinary ketosteroids in females

Values for females deserve examination, since a) animal ovarian tissues contain androgens (61); b) in normal female animals the ovarian secretions stimulate and maintain the growth of prostatic rudiments and other secondary sexual organs of the male type (62, 63); c) ovarian secretions can exert even stronger androgenic effects if the ovaries are transplanted to a cool environment, as demonstrated in mice (64) and rats (65); and d) certain types of ovarian tumors can cause virilism in women (66).

Table 2 and Figure 1 show that the index changes with age in the same general fashion in females as in males. The index was found to be 0.5 in 11 girls 2 to 8 years old, 2.0 in 8 adolescent girls 13 to 15 years old, and 2.8 in 35 young adult females. Decrease to a mean value of 2.0 was noted in 26 women 40 to 54 years of age and to 1.8 in 5 women 55–75 years old.

In 11 ovariectomized women the index was lower than in normal young women (2.1 *vs.* 2.9) but not as low as the 1.4 observed in eunuchs.

DISCUSSION

Testicular secretions in humans decrease progressively and materially with advancing age. This has been demonstrated by quantitative measurements of a secondary sex character, axillary hair, which in men requires testicular secretions for its maintenance (56). The present analysis provides further evidence. Androgenic titers are 69 per cent lower in old men than in young men (Table 1); since more than two thirds of the urinary androgenic activity in normal young men originates from testicular secretions (Figs. 2 and 3), it must be concluded that testicular function diminishes in aging men. This decrease in urinary androgenic titers from young (20 to 39 years) to old age (60 to 75 years) is 90 per cent as large as that observed after complete elimination of testicular secretions by surgical means (Table 1).

There is much additional evidence that testicular secretions decrease upon aging, resulting in a) a smaller proportion of urinary ketosteroids derived from testicular secretions, and b) less androgenic activity per mg. of colorimetrically determined ketosteroids. A marked decrease with age has been observed by us in the androgenicity of the alpha fraction (15). The mean was 43 I.U. per day in 16 men 21 to 39 years of age, but only 7 I.U. in 7 men 60 to 75 years of age. As studied in young men, 90 per cent of the androgenicity of the alpha fraction was found to be due to androsterone (Fig. 2), a compound derived chiefly from testicular precursors (4). Therefore, emphasis should be placed upon the great reduction with age in the number of I.U. of androgenic activity per mg. of colorimetrically determined ketosteroids in the alpha fraction. This value decreased from 4.2 in the 21 to 39 year-old men to 1.8 in the 60 to 75 year-old men (15), a decline with age that is significant at the level of 1 per cent.

Although not as pronounced, a similar trend in the direction of decreased testicular secretions is seen in studies of urinary ketosteroids, employing isolation procedures.[5] Per 24-hour sample of urine, a mean of 3.3 mg. of androsterone was isolated in 10 men 21 to 35 years of age, who excreted 14.5 mg. of alpha-ketosteroids per day. A mean of only 1.3 mg. of androsterone was isolated from 6 men 61 to 76 years old, who excreted 7.0 mg. of alpha-ketosteroids daily.

Histologic evidence that testicular secretions diminish with age is a decrease in the lipid content of the interstitial cells of Leydig (67), which are the presumed site of androgen production.

Indirect evidence that testicular secretions slacken with age in the presence of greatly supranormal titers of urinary gonadotropins in about 14 per cent of 29 apparently normal men[6] 50 or more years of age (68). Titers of gonadotropins are known to increase in some subjects if gonadal secretions decrease (69).

It is theoretically possible that the percentage of androsterone derived per unit of secretion by the testes changes with age,[7] but no evidence for a major shift in this regard was observed in the studies at the Sloan-Kettering Institute[5]. Comparisons of amounts isolated from the urine of the two chief metabolites of testicular secretions, namely the strongly androgenic androsterone and its practically nonandrogenic isomer, etiocholan-

[5] These data (4, 40) were made available through the kindness of Dr. Thomas Gallagher of the Sloan-Kettering Institute.

[6] Some of these men are the same subjects whose titers of ketosteroids (14) and androgens (15) are listed in Table 1.

[7] In subjects with increased testicular secretion as a result of interstitial-cell tumors of the testes (17, 18) the ratios of androsterone to etiocholanolone seem to have been extremely high.

olone, showed almost similar ratios in 10 young men (42:58) and 6 old men (39:61). This finding does not exclude the possibility that testicular secretions are metabolized to form less etiocholanolone as well as less androsterone in old men than in young men. Such an hypothesis can be tested by ascertaining if, prior to inactivation or other metabolic alteration, the testicular secretions maintain the growth of axillary hair in a flourishing state in the aged. That this secondary sex character reflects the influence of testicular secretions, and their diminution, is shown by its atrophic state or complete absence in eunuchs (56). Those androgens which are present would be expected to continue to exert effects in elderly subjects, as shown by studies of the metabolic effects produced by the administration of testosterone proprionate to old men (58). In one series of men (14, 15), whose ketosteroid values are included in Table 1, the mean weight of the full-grown mass of axillary hair was only 53 mg. in 8 men 60 to 75 years old as compared with 143 mg. in 18 men 21 to 39 years of age. This points to a decrease in testicular secretions with aging and not to altered metabolism of unchanging amounts of secretions.

There is strong evidence that extra-testicular as well as testicular contributions of precursors of urinary androgens decrease with age despite claims (70) that adrenocortical secretions undergo practically no decrease with age insofar as can be judged by values for urinary neutral lipids. The secondary sex character, axillary hair, which depends on testicular secretions for its maintenance (56), had an average weight of 53 mg. in men 60 to 75 years of age,[6] which is about three times the values of 7–20 mg. found in eunuchs (56). Thus, testicular secretions are maintained to some extent in old men and these secretions must be assumed to be contributing precursors to urinary ketosteroids. Inasmuch as Hamburger (12) has shown that values for total urinary ketosteroids in 21 men 60 to 75 years old (5.3 mg.) are similar to those found in 10 eunuchs 28 to 52 years of age (5.6 mg.)—although the testes are contributing to urinary ketosteroids in old men—the extra-testicular contributions must be presumed to be decreased.

Further evidence of decreased adrenocortical secretions in aging men is the decreased androgenic titers of the beta fraction in old men (15). The androgenic activity of this fraction owes almost entirely to dehydroisoandrosterone (Fig. 2), a compound derived from adrenocortical secretions (29, 51, 59, 60).

Isolation studies carried out at Sloan-Kettering Institute[5] also indicate that aging men excrete smaller amounts of two urinary alpha-ketosteroids which are metabolites of adrenocortical secretions. Per twenty-four hours, an average of 0.68 mg. of 11-ketoetiocholanolone was excreted by 6 men 61 to 76 years of age, as compared with 0.92 mg. by 10 men 21 to 35 years

old. The old men excreted 0.92 mg. of 11-hydroxyandrosterone, the young men 1.21 mg.

The decline with age in excretion of urinary ketosteroids derived from precursors contributed by extra-testicular sources (to a great extent by the adrenal cortices) is, however, less drastic (15) than the decline in ketosteroids from precursors chiefly of testicular origin. This phenomenon is also evident in the isolation studies carried out in the Sloan-Kettering Institute in which the percentage of total ketosteroids which were metabolites from extra-testicular sources was actually greater in old as compared with young men for both 11-ketoetiocholanolone (7.8 *vs.* 5.2 per cent) and 11-hydroxyandrosterone (10.6 *vs.* 6.9 per cent). In contrast, the relative percentage of total ketosteroids due to metabolites chiefly from testicular secretions was less in old than in young men —14.8 *vs.* 19.0 per cent for androsterone, and 23.1 *vs.* 26.1 per cent for etiocholanolone.

Obesity is accompanied by higher titers of urinary ketosteroids and androgens, to an extent detectable until at least the sixth decade of life (71). Each kilogram of body fat augments the daily titers of urinary ketosteroids in men 21 to 39 years old, by approximately 0.5 mg. in colorimetric assay and 3.2 I.U. in androgenic values. The degree of obesity does not distort the index described in the present account, however (15). Apparently the deposition of body fat does not influence testicular contributions to urinary ketosteroids to any greater extent than it does the precursors from extra-testicular sources, and hence does not interfere with the use of the index as a measure of the proportions of testicular *vs.* extra-testicular secretions.

The fact that indices lower than those in eunuchs are observed in young children and in certain cases of pituitary insufficiency and hypothyroidism indicates that in these states the androgenic activity of ketosteroids is decreased more than could be accounted for by loss of testicular secretions. Contributions by extra-testicular as well as testicular sources must be diminished under these circumstances. Under such conditions the index would not seem to be serviceable in gauging the relative contributions of testicular and extra-testicular sources to the moiety of urinary ketosteroids.

The index does not appear to be as useful in females as in males in differentiating between the relative contributions of gonadal and extra-gondal sources. Apparently the ovarian secretions do not influence the index to the same extent as testicular secretions. The index never attains as high a value in young women as in young men, and bilateral oöphorectomy does not lower the index to the extent observed following bilateral orchiectomy.

It remains to be shown if the index possesses any important utility in clinical studies that will make it worthwhile to carry out androgenic assays which, with current techniques, are laborious and lack precision (72). Perhaps the guiding principles which underlie the use of this index can be utilized more efficiently in comparisons of the amounts of individual steroids isolated from urine by chromatographic or other techniques.

A question deserving of consideration is whether or not this index will provide information of value in certain types of prostatic and mammary cancer which remain sensitive to their endocrine environment.

SUMMARY

It is necessary to dispute the almost universally prevailing viewpoint that a colorimetric assay of extracts of urinary ketosteroids reliably gauges the androgenic activity of this mixture of compounds. Actually, the androgenic activity per mg. of colorimetrically measured ketosteroids in urinary extracts differs with age, sex, and the clinical state of the subject.

In normal males an index, based on the number of I.U. per mg. of colorimetrically measured urinary ketosteroids, is high (3.7) when testicular secretions are at their peak, which is in young adults. Lower values are found in immature children (0.2), adolescents (1.3–2.9), middle-aged men (3.4), old men (2.9) and eunuchs (1.4). Unusually high values appear to have existed in subjects with supranormal testicular secretions as a result of interstitial-cell tumors of the testis. The average number of I.U. per mg. of urinary ketosteroids is 28 per cent higher in young men than in young women (3.7 *vs* 2.9).

The utility of this index has a chemical basis. Metabolites from testicular secretions produce high values for androgenic activity per mg. of ketosteroids, because they include a large proportion of androsterone, a compound which has by far the highest androgenic activity of any ketosteroid found in human urine. The metabolites derived from extra-testicular sources are present in a larger quantity than androsterone but they possess less androgenic activity.

With this index, an estimation may be made of the proportions of urinary ketosteroids derived from testicular secretions versus those from extra-testicular sources. Thus the index may be employed to evaluate the respective degrees of endocrine function of testicular and extra-testicular sources.

Testicular secretion of precursors of urinary ketosteroids decreases with age. Precursors contributed by extra-testicular sources, which appear to be chiefly the adrenal cortices, also decrease with age but to a much less drastic extent.

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STEROID STUDIES. II. THE METABOLISM OF ANDROSTAN-17(β)-OL-3-ONE*

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IN AN earlier paper (1) some effects of androstan-17-(β)-ol-3-one (stanolone) in aged people were described. The results of a chromatographic study of the metabolism of stanolone by an agonadal male are now presented.

PROCEDURE

A 43-year-old obese male, who on physical examination showed a split-pea sized mass in each scrotum, was selected for this study. Both of these masses were subjected to biopsy. No functioning testicular elements—spermatogonia, Sertoli cells, or interstitial cells of Leydig—could be identified. At the age of 13, the patient was a normally developed individual with both testes readily palpable in the scrotum and of normal size and consistency. Following an accident at that time, both testes underwent apparently complete atrophy and there were consequent alterations in growth and sexual development.

In our study, this subject was given for forty-five days a constant daily diet which contained 800 calories, with an average content of 60 Gm. of protein, 24 Gm. of fat and 89 Gm. of carbohydrate. From the thirtieth through the forty-first days, a daily dose of 50 mg. of stanolone was administered by intramuscular injection. From the twenty-sixth through the forty-fifth day, aliquots of food for each 48-hour period were analyzed for sodium, potassium, chloride and nitrogen and two-day pools of urine were analyzed for sodium, potassium, chloride, nitrogen and 17-ketosteroids (Table 1).

Blood was analyzed for sodium, potassium, chloride, total cholesterol, total serum protein, albumin and globulin at the end of the twenty-ninth, forty-first and forty-fifth days. No change was found in the concentration of any of these substances.

Before beginning the intramuscular injections of stanolone all the urine excreted for a period of three days was pooled and the urinary 17-ketosteroids were extracted (2). An aliquot of the extract was chromatographed on alumina[1] by the procedure of Wilkins and Carlson (3). It took from fifteen to twenty-five minutes for 25 ml. of eluant to pass

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[1] Adsorption grade alumina obtained from Fisher Scientific Company, New York.

TABLE 1. ANALYSES OF URINE

Day of study	Two-day differences (intake* minus urinary excretion)				Ketosteroids; 48-hour excretion (mg.)
	N (Gm.)	Na (mEq.)	K (mEq.)	Cl (mEq.)	
26, 27	−6.8	+43	+14	+25	15.6
28, 29	−9.2	−25	+ 2	−10	12.2
30, 31†	−8.5	+18	+26	+17	19.1
32, 33	−7.8	−24	+38	− 5	24.9
34, 35	−1.0	−10	+35	−22	37.6
36, 37	−4.0	−14	+26	−27	37.7
38, 39	−3.0	−64	+18	− 8	35.6
40, 41‡	−4.0	−36	+19	−14	40.8
42, 43	—	—	—	—	—
44, 45	−5.0	−55	+28	−21	60.3

* Average two-day intakes were:
Nitrogen 19.5± 1.2 Gm.
Sodium 99.2±25.9 mEq.
Potassium 82.6± 5.8 mEq.
Chloride 101.5±24.8 mEq.
† First two days of injection of steroid.
‡ Last two days of injection of steroid.

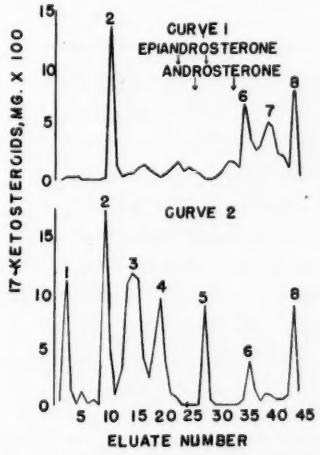

FIG. 1. Chromatographic pattern of urinary steroids excreted before (Curve 1) and during (Curve 2) the period of injection of stanolone.

through the column. The fractions obtained chromatographically were assayed for 17-ketosteroids by a modified Zimmermann procedure (4) (Fig. 1, Curve 1). In the same way, the total urinary neutral 17-ketosteroids excreted from the twenty-fourth through the fortieth day of the study, were extracted, chromatographed and assayed (Fig. 1, Curve 2). Curve 1 represents a total of 0.71 mg. of 17-ketosteroids or 8.9 per cent of the "pre-stanolone" 24-hour urinary 17-ketosteroid excretion. Curve 2 represents 1.32 mg. or 8.9 per cent of the "post-stanolone" 24-hour urinary 17-ketosteroid excretion. Any fraction shown in Curve 2 should contain at least as much 17-ketosteroid material as the corresponding fraction in Curve 1, if there has been no change in the basic level of endogenous 17-ketosteroid formation. Pooled urines were studied in order to minimize the effect of daily variations in 17-ketosteroid excretion.

TABLE 2. SEQUENCE OF ELUTION OF CRYSTALLINE 17-KETOSTEROIDS FROM ALUMINA

17-Ketosteroid		Fractions in which steroid was eluted
Name	Mg.	
Dehydroepiandrosterone acetate	0.10	6–10
Dehydroepiandrosterone	0.10	21–26
Epiandrosterone	0.10	22–27
Androsterone	0.10	25–32
Etiocholanolone	0.10	33–35
Androstanolone	0.10	43–45

For comparison, solutions of known ketosteroids[2] were also chromatographed. The order of elution of these steroids (Table 2) from the alumina column was the same as that recorded by Dingemanse *et al.* (5, 6), Lieberman *et al.* (7) and Wilkins and Carlson (3).

RESULTS AND DISCUSSION

Stanolone had effects upon the nitrogen balance (Table 1) similar to those previously described (1). After the steroid had been given for four days, there was a decrease in the patient's loss of nitrogen, accompanied by a decrease in the rate of loss of weight.

Because of the large variation in the urinary excretion of sodium, potassium and chloride before the first injection of stanolone was given, no statistical significance could be attached to our results. However, there seems to have been a trend towards an increase in the urinary excretion of sodium beginning on the ninth day after stanolone was first given, and a decrease in the urinary excretion of potassium immediately after the injections of stanolone were started. The urinary excretion of chloride

[2] Crystalline ketosteroids were supplied by Dr. E. L. Henderson, Schering Corp., Bloomfield, N. J., Dr. J. R. Jewel, Ayerst, McKenna and Harrison, Ltd., New York. N. Y., and Dr. Irving Sollins, Chemical Specialties Co., Inc., New York. N. Y.

seemed to increase but the trend was not as evident as in the case of sodium.

The chromatographic "spectrum" of the total 24-hour urinary neutral 17-ketosteroids excreted before stanolone was administered showed four distinct peaks (Fig. 1, Curve 1, peaks 2, 6, 7 and 8). After stanolone was injected, seven peaks were found (Fig. 1, Curve 2, peaks 1, 2, 3, 4, 5, 6 and 8). Peaks 1, 3, 4 and 5 were not present in Curve 1 and the excretion of the ketosteroids in the chromatographic fractions 9 through 11, or peak 2, had increased. This increase in ketosteroid excretion represents, at least in part, the excretion of metabolites of stanolone, a steroid with the same chemical structure as testosterone except for the absence of a double bond between carbons 4 and 5. Because epiandrosterone and androsterone are metabolites of testosterone (8, 9), it might be expected that they also would be metabolites of stanolone. When solutions of crystalline preparations of these steroids were chromatographed, epiandrosterone and androsterone appeared in the region of peak 5, and stanolone appeared in the region of peak 8 (Table 2). Therefore, the increase in peak 5 may indicate that epiandrosterone and/or androsterone are metabolites of stanolone.

The decrease in the ketosteroids in fractions 29 through 41, after the injection of stanolone, indicates a suppression of endogenous production of 17-ketosteroids.

The general assumption in metabolic studies which involve urinary steroids has been that the administration of an 11-desoxy-17-ketosteroid does not alter the basic level of endogenous steroid production, although West and coworkers (10) did suggest that perhaps intramuscular injections of large amounts of testosterone propionate might depress endogenous formation of steroids. Our results, which indicate that stanolone did depress the formation of ketosteroids or their precursors by the adrenal cortex in the agonadal male, show that the assumption of a constant basic level of endogenous steroid formation is not necessarily valid.

Peak 8, the region in which stanolone appears when chromatographed (Table 2) was not significantly affected by the steroidal treatment, indicating that stanolone was not excreted as such in the urine. However, the possibility must be considered that an increase in peak 8 due to the excretion of stanolone may have been negated by a decrease in endogenous formation of steroids.

SUMMARY AND CONCLUSIONS

1. Some metabolic effects of daily intramuscular injections of 50 mg. of androstan-17(β)-ol-3-one (stanolone) in a 43-year-old agonadal male have been described. Trends were observed which indicated that 50 mg. of

stanolone daily decreased the urinary excretion of potassium, and increased the urinary excretion of sodium and chloride. The urinary excretion of nitrogen was decreased and that of neutral 17-ketosteroids was increased.

2. Neutral urinary 17-ketosteroids excreted before and during the period of administration of stanolone were chromatographed and evidence for the conversion of stanolone into epiandrosterone and androsterone was obtained.

3. Stanolone appeared to depress the endogenous formation of some neutral 17-ketosteroids.

4. The injected stanolone probably was not excreted as such in the urine.

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Teaching Clinic

MYXEDEMA HEART

A CASE REPORT

THE following patient,[1] presenting the clinical picture of myxedema with extensive anasarca, was of great interest in our understanding of the problem of "myxedema heart":

S.S., a 30-year-old single, Jewish advertising manager was admitted to the Massachusetts General Hospital on February 4, 1952, because of progressive swelling of the feet and ankles. At the age of 21 it was discovered that he had diabetes mellitus and he was treated by means of dietary regulation and insulin. Supervision was difficult and the patient failed to test his urine regularly. He adjusted the daily dose of insulin according to his own feelings, usually taking 18 units of protamine-zinc insulin and 12 units of regular insulin. One year before admission swelling of the ankles and anemia developed. He was treated by his local doctor, following which the edema disappeared and the blood returned to normal. Three months before admission, anemia and progressive swelling of the feet and ankles again developed. This time, treatment similar to that given previously was without benefit. He complained of weakness, inability to climb stairs, extreme sensitivity to cold, constipation, dry skin and loss of libido. There was no history of blood loss, except slight bleeding from the gums; dyspnea, palpitation, precordial pain and neurologic symptoms were absent. He liked salt and consumed a good deal of it. He had gained 20 pounds in three months.

Past history: There was no history of scarlet fever or of symptoms of renal disease.

Family history: His mother had had diabetes and had died of cancer of the bowel at the age of 42.

Physical examination: He was found to be lying flat in bed, comfortable, pale, slightly drowsy, and showing generalized edema including the eyelids, face, arms, feet and legs. His weight was 172 pounds. His speech was slightly slurred and thick. His skin was dry, cool, coarse and scaly, and his hair was coarse and sparse. The right pupil was greater than the left and both reacted well; there was minimal vascular narrowing and A-V nicking in the fundi. The neck veins were flat and there were no masses. The heart was enlarged to the left, sounds were regular with presystolic gallop in the mitral area but no murmurs, and the blood pressure was 114/74. The lungs showed evidence of fluid at both bases. There was a moderate amount of ascites and there was massive edema of the extremities up to the knees. The gonads were of normal size and consistency. Neurologic examination showed active reflexes of the upper extremities with slow relaxation of contractions, but absent ankle jerks.

[1] I am indebted to Dr. Bernard M. Jacobson of the Massachusetts General Hospital and Dr. Louis Kunian of Lynn, Massachusetts, for allowing me to participate in the management of this patient and for permission to publish the data.

Laboratory findings: Soon after admission, the following procedures were carried out: Urine—specific gravity 1.024, no albumin, 2+ sugar, normal sediment. Blood—W. B. C. 12,800; hemoglobin 11.2 grams per 100 ml.; normal differential count; normal platelet count; smear showed slight hypochromia and slight variation in size and shape of cells with an occasional elongated, tailed or oval form; hematocrit 33; sedimentation rate 40 mm. per hour; chemical analysis showed blood sugar concentration 67 mg. per 100 ml., nonprotein nitrogen 25 mg., cholesterol 232 mg., total protein 6.54 Gm. (albumin 4.0 Gm., globulin 2.54 Gm. and A/G ratio 1.6), calcium 8.5 mg., phosphorus 3.5 mg. per 100 ml., CO_2 35.8 mEq. per liter, sodium 132.2 mEq., chloride 93 mEq. and potassium 3.3 mEq. per liter.

The initial examiner wrote, "No diagnosis is possible. Edema is too extensive to be myxedema. Renal origin, in absence of albumin, is unlikely." Another examiner wrote, "primary myxedema most likely."

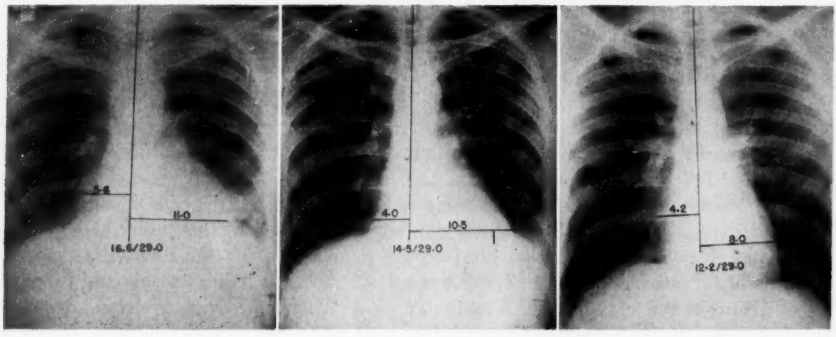

FIG. 1. On the left is the x-ray appearance of the heart and lungs on admission. The middle photograph shows changes within ten days, during rest and a low-salt diet. The photograph on the right shows the further changes after nine months of thyroid medication.

On the day after admission a roentgenogram of the chest (Fig. 1) showed the heart to be enlarged in all diameters, the pulsations of the borders of the heart to be diminished, considerable fluid in both pleural spaces, and the hilar and pulmonary vascular shadows to be somewhat increased in prominence. An electrocardiogram showed normal rhythm, rate 70 per minute, PR interval 0.2 second, low T_1, T_2 and T_3, low T in V_2 to V_5, flat T in V_1 and V_6 and small QRS complexes. Excretion of phenolsulfonphthalein was normal, *viz.*, 25 per cent in fifteen minutes, 15 per cent in thirty minutes, 20 per cent in one hour and 10 per cent in two hours—a total of 70 per cent. Circulation time (arm to tongue) was twenty-three seconds, vital capacity 3.4 liters (75 per cent) and venous pressure 115 mm. of water. A bromsulfalein test for liver function showed 4 per cent retention. The initial BMR was −20 per cent.

The writer saw this patient three days after admission and wrote as follows: "Characteristic primary myxedema. The other endocrine glands are normal. The loss of libido and erections is due to myxedema and is not gonadal in origin. I would suspect his myxedema to be of three to four years' duration. The degree of edema is unusual and may, in some way, be related to the diabetic state but more likely to the longstanding myxedema . . . the diagnosis of myxedema and 'myxedema heart' will explain the entire clinical picture. I predict that thyroid medication will rid him of everything except the diabetes."

Clinical course: During chair and bed rest, a moderately low-salt diet and a diabetic regimen, this patient had a brisk diuresis and lost 27 pounds in ten days. Further laboratory studies confirmed the diagnosis of myxedema—the thyroidal radioactive iodine uptake was 3.8 per cent, the protein-bound iodine concentration in the blood 1.1 gamma per 100 ml., and the BMR −36 per cent. Re-examination of the chest by x-ray (Fig. 1) showed a decrease in the transverse diameter of the heart, considerable clearing of congestive changes in both lungs, and a small amount of fluid on the left but none on the right. Because the blood sodium level had dropped to 123 mEq. per liter the patient was started on an extra 5 grams of salt per day. In addition he was given sodium *l*-thyroxine orally, 0.2 mg. twice daily. During this regimen he improved considerably and at the end of two weeks he was much more alert, his speech was clearer, the puffiness of his face and the peripheral edema had disappeared and he had lost another 10 pounds. His BMR reached a level of −11 per cent and the blood cholesterol concentration dropped to 157 mg. per 100 ml. As expected, his insulin requirement increased with the administration of thyroid hormone. The electrocardiogram taken before discharge showed that the T waves and the QRS complexes had returned to the normal pattern.

When seen nine months later, he was taking 100 mg. of thyroid daily, and was completely free of myxedema and of cardiac symptoms. X-ray examination of the chest (Fig. 1) showed further shrinkage in the size of the heart and complete clearing of the lungs.

DISCUSSION

In a previous communication we presented our experience with "myxedema heart" (1) and came to the conclusion that the condition described by Zondek (2) (dilatation of the heart, slow indolent heart action, and low complexes in the electrocardiogram) is common, whereas the condition described by Fahr (3) (cardiac failure) is uncommon, and the symptoms only slight when present. A mild degree of generalized edema and serous effusion is common in myxedema and can be accounted for by the high capillary permeability described by Lange (4). The patient just described presented an unusual degree of anarsarca—to a degree we had never seen in myxedema. Could this be due principally to congestive failure, as maintained by Mussio-Fournier and associates (5)? It was the opinion of most observers who saw this patient that congestive heart failure alone could not account for the massive edema, but that myxedema plus some other factors could account for it. The factors, in this case, were twofold, *viz.*, a mild degree of decompensation plus a high intake of salt. The latter was volunteered by the patient; the former was suggested by the pulmonary congestion seen in the x-ray film of the chest. Favoring this view farther was the loss of 27 pounds and considerable shrinkage of the heart under a regimen of no special medication other than a low-salt diet and rest. However, the continued improvement in the clinical condition, including the cardiac state, with the administration of moderate doses of thyroid hormone, plus the absence of underlying heart disease and a normal blood pressure, indicated that "myxedema heart" was the most important single factor.

There have been numerous reports in the literature of pericardial effusion developing in patients with myxedema, and the suggestion has been made that pericardial effusion accounts for many of the findings in "myxedema heart." A recent report by Marks and Roof (6) presents this point of view, with a summary of the literature. Although one cannot be certain that our patient did not have a significant amount of pericardial effusion, the evidence is against it, particularly the absence of signs of tamponade, and the diminution in anasarca and shrinkage in cardiac size with only rest and limitation of salt intake.

SUMMARY

A case history is described of a young man with myxedema of several years' duration, presenting massive edema, ascites and bilateral pleural effusion. His condition became normal during rest, limitation of salt intake and administration of thyroid hormone. It was concluded that the clinical picture could be accounted for predominantly by the physiologic changes produced by "myxedema heart," and only partially by the presence of mild congestive heart failure and the consumption of large amounts of salt.

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Editorial

DIABETES AND GROSS LESIONS OF THE PANCREAS

THE occurrence of diabetes mellitus as a manifestation of neoplastic or inflammatory disease of the pancreas is frequently thought of as being so unusual as to be without much diagnostic or practical importance. The concurrence of the two conditions is, of course, rare when compared with the incidence of a disease as common as diabetes, but when considered in relationship to the incidence of pancreatic lesions, it is by no means uncommon. Whereas this association has long been recognized as one of singular historical interest, available data suggest that it is important enough to be of practical concern as well.

In 1788, Thomas Cawley called the attention of his colleagues to "a singular case of diabetes" in which the pancreas had been destroyed by calculous obstruction (1). He speculated on the cause of diabetes, reviewing theories which existed at that time but, believing the disease to be primarily one of the kidneys, did not actually associate the unusual manifestations in the pancreas with the pathogenesis of the disorder. An analogous situation with respect to neoplasm was first recorded by Richard Bright, who in 1833 reported the case of "James Barnes, aged 49, clerk in a waggon office, a man of sober habits" who "began to complain in March 1827 of immoderate thirst and appetite with a constant pain in his loins" (2). The report proceeds to tell that the unfortunate man became jaundiced in September of the same year, and twelve months following the onset of diabetes died of what appears from Bright's postmortem description to have been carcinoma of the head of the pancreas. During the years which followed numerous similar cases were reported. Even Claude Bernard mentioned "in passing" 2 cases of diabetes associated with extensive atrophy of the pancreas (3). He apparently thought little of the actual relationship between these two conditions, for it remained for Lancereaux to introduce the concept of pancreatic diabetes after studying intensively 2 cases of lithiasis (4). More than forty years later pancreatic lithiasis was again to play an historical role, for it was from Barron's discussion of the pathologic alterations in that disease that Banting conceived the principle of extracting degenerated glands after ligature of the pancreatic ducts (5).

Dr. Moore, who has recently reviewed the cases of carcinoma of the

pancreas in the University of Minnesota Hospitals, found that in 22 of 193 cases (11.8 per cent) the additional diagnosis of diabetes was made (6). As in all such series, the time relationship between the development of a pancreatic lesion and the metabolic disturbance was frequently obscure, but in a number of instances there was an impressive correlation between the onset of symptoms of the tumor and the appearance of evidence for diabetes. The incidence he found is in the same range as that which others have reported (Silver and Lubliner, 13.4 per cent; Mikal and Campbell, 13 per cent) (7, 8).

Even in the absence of diabetes of sufficient severity to cause symptoms or require treatment, an elevated blood sugar level or decreased glucose tolerance are present in a significant number of patients with pancreatic carcinoma. For such manifestations Marble reported an incidence 12.9 per cent, Berk, 19.4 per cent and Broadbent and Kernan, 33 per cent (9, 10 11). Dashiell and Palmer found glycosuria in 27.1 per cent and abnormal glucose tolerance curves in 18 of 21 patients in whom the tests were performed (12). The importance of investigating carbohydrate tolerance is intensified by the fact that the occurrence of such abnormalities is most frequent in lesions of the body and tail, the diagnosis of which is notoriously difficult.

We have recently studied the findings in 28 patients in whom the diagnosis was "chronic pancreatitis," "chronic suppurative pancreatitis," "chronic relapsing pancreatitis," "pancreatic lithiasis" or "chronic calcareous pancreatitis." Seven of these (25 per cent) had diabetes requiring treatment. This incidence is almost exactly that reported from the Mayo Clinic on the basis of a similar group of patients (13). Still more striking was the finding that among the 7 of our patients in whom pancreatic calculi were demonstrable, clinical diabetes existed in 4. We would concur therefore with Cattell and Warren who state: "One-third of patients with relapsing pancreatitis observed by us had diabetes. The appearance of diabetes in a patient who complains of chronic progressive epigastric pain should suggest the possibility of chronic pancreatitis" (14).

Diabetes developing during or in the wake of acute pancreatitis is also not rare (15). In our experience the manifestations are usually transient and disappear with the subsidence of an acute attack, but permanent diabetes on this basis is by no means unknown (16). Though the occurrence of glycosuria under these circumstances is considered to be a grave prognostic sign, one wonders whether the poor outlook may not be partly associated with delay in recognition and treatment of the lesion. The author was impressed with this circumstance recently in a case of postoperative pancreatitis in which the etiology of severe polyuria was overlooked for a time, because parenteral glucose solutions were being administered and a degree of glycosuria was not felt to be abnormal.

The association between diabetes and gross lesions of the pancreas is therefore of obvious practical as well as historical importance. It may occasionally be of great diagnostic value in a field in which more specific diagnostic procedures are unfortunately lacking.

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Questions and Answers

(Questions submitted at the 1953 Postgraduate Assembly of the Endocrine Society and the University of Minnesota)

Answers by Fuller Albright, Massachusetts General Hospital, Boston, Mass.

QUESTION: Do patients with osteoporosis ever have hypercalcemia?

ANSWER: Yes, patients with osteoporosis can have hypercalcemia. This is most often met in cases of osteoporosis associated with acute disuse atrophy *e.g.*, poliomyelitis.

QUESTION: How do you explain the localized osteoporosis which may occur with hypervitaminosis D?

ANSWER: I do not recognize osteoporosis as secondary to hypervitaminosis D. The second action of vitamin D, *viz.*, to increase the urinary phosphorus excretion, could lead to demineralization, but I would expect this to be on the basis of an osteitis fibrosis.

QUESTION: Do estrogens and androgens cause bone formation in other types of osteoporosis, in addition to the postmenopausal type?

ANSWER: The evidence to date is that they do.

QUESTION: Is there any relationship between the occurrence of menopausal symptoms and osteoporosis in postmenopausal women? What bearing does this have on therapy?

ANSWER: I do not think that the presence or absence of menopausal symptoms (such as hot flashes and depression) has any effect on the development of postmenopausal osteoporosis. It follows that the treatment of postmenopausal osteoporosis should not be governed by symptoms, other than those originating in the skeleton.

QUESTION: Does the alkaline phosphatase level in postmenopausal osteoporosis rise with steroid therapy?

ANSWER: No, the alkaline phosphatase level does not rise with steroid therapy in cases of postmenopausal osteoporosis. In "steroid therapy" I include estradiol, stilbestrol and testosterone.

Answers by Harold L. Mason, Mayo Clinic, Rochester, Minn.

QUESTION: Please discuss the origin of acid phosphatase. Is it derived from the cancer cells? Is the rise in the alkaline phosphatase level following stilbestrol therapy for cancer of the prostate due to increased osteoblastic activity?

ANSWER: The evidence indicates that in cancer of the prostate the serum acid phosphatase comes from the cancer cells. In cases without bone metastases the serum acid phosphatase level is increased only in about 25 per cent of cases, whereas in cases with bone metastases the activity is increased in about 80 per cent of cases. It is not evident why the serum acid phosphatase activity should be increased more often in the cases with bone metastases, as the activity in the neoplastic tissue does not appear to be increased. It may be that there is some change in the character of the cells of the metastatic lesions which permits a more ready diffusion of the enzyme into the blood.

The increase of alkaline phosphatase activity following therapy for cancer of the prostate is associated with increased osteoblastic activity and laying down of new bone. The osteoblasts are rich in alkaline phosphatase and presumably are the source of the increased enzyme activity in the blood.

QUESTION: Please discuss the Allen test. Does dehydroisoandrosterone occur in normal urine?

ANSWER: The original description of this test by Allen, Hayward and Pinto (1) gives a good account of its advantages and the possible interference by substances other than dehydroisoandrosterone. It was proposed by these authors that this simple color test should be of considerable help in distinguishing between the presence of an adrenocortical tumor and the presence of adrenocortical hyperplasia. It is generally true that the proportion of dehydroisoandrosterone (or dehydroepiandrosterone) which is detected by this method is greatly increased in cases of adrenocortical tumor, although a number of cases have been reported in which the total excretion of 17-ketosteroids and of the β-fraction (another test for dehydroisoandrosterone) were within normal limits. Some caution should be used in the interpretation of any test which is alleged to be specific for dehydroisoandrosterone, since it has become evident that other substances may give rise to the same color reaction (2). Dehydroisoandrosterone does occur in normal urine.

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Answers by Joseph W. Jailer, Columbia Univ. College of Physicians & Surgeons, New York, N.Y.

QUESTION: Decholin (bile salts) has been used intravenously for treatment of serum sickness and other hypersensitivity states. Have you had any experience with this drug?

ANSWER: We have had no personal experience with this therapy. A combination of bile salts has been used in rheumatoid arthritis but, as far as I know, the results are not very decisive.

QUESTION: Is there any danger of precipitating psychosis by the use of cortisone in Addison's disease?

ANSWER: The incidence of abnormal mental states in untreated Addison's disease is fairly high (Cleghorn, R. A.: *Canad. M.A.J.* **65**: 449, 1951). The introduction of cortisone in the treatment of adrenal insufficiency has led to an improvement in the psychologic status of patients with Addison's disease. However, mental excitement sometimes does occur early in treatment, even with small doses of cortisone, but quickly subsides (Cleghorn, R. A.: *J. Clin. Endocrinol. & Metab.* **13**: 1294, 1953).

It should be borne in mind that the dosage of cortisone required to maintain an Addisonian patient is very much smaller than that needed to induce a remission in other diseases. In Addison's disease, one attempts to make a hypoadrenal patient normal, whereas in other diseases, the "hyperadrenal state" must be achieved. Here again, the incidence of psychologic changes is high.

QUESTION: Is "stress" a factor in the etiology of collagen diseases or of any disease process? If so, how?

ANSWER: It is not known whether stress plays any role in the etiology of "collagen" diseases in the human. As you know, Dr. Selye has proposed a theory to that effect. Some of the pros and cons are discussed in a symposium which may be found in Recent Progress in Hormone Research, Vol. VIII, pp. 117–272.

Answers by Robert H. Williams, Univ. of Washington School of Medicine, Seattle, Wash.

QUESTION: What is the danger of agranulocytosis continuing during Tapazole administration, when agranulocytosis has occurred during propylthiouracil administration?

ANSWER: Some patients who have had agranulocytosis during therapy with propylthiouracil have not experienced it when subsequently given Tapazole, and vice versa. On the other hand, it has occurred in certain patients during the administration of each drug; in such instances it is advisable not to give either Tapazole or propylthiouracil until the agranulocytosis has disappeared. In some patients it is best to change to iodide therapy temporarily; in some, radioiodide therapy or subtotal thyroidectomy may be offered, without resumption of antithyroid drugs.

QUESTION: If iodinated propylthiouracil is effective for rapid preoperative preparation even when potassium iodide and propylthiouracil fail, why not use it routinely for surgical preparation?

ANSWER: Iodinated thiouracil rapidly promotes euthyroidism in some patients but not in others; it is not possible to predict the response in an individual patient. Iodothiouracil fails much more often than does a combination of potassium iodide and propylthiouracil and this is the chief reason for the less frequent use of iodinated thiouracil.

QUESTION: Why is amenorrhea present in acromegaly? Why diabetes? Why the increase in the level of serum phosphorus?

ANSWER: In acromegaly, amenorrhea may be due to alterations in the amount and relative proportion of the gonadotropins, and an increase in androgens. Diabetes results from the antagonistic effect of growth hormone on the action of insulin and from pancreatic islet-cell exhaustion; in some instances there is also hyperadrenocorticism. The increase in the level of serum phosphorus has not been adequately explained.

The Endocrine Society

THE 1954 ANNUAL MEETING

The Thirty-sixth Annual Meeting of The Endocrine Society will be held n the Sir Francis Drake Hotel, San Francisco, California, Thursday, Friday and Saturday, June 17–18–19, 1954.

Dr. Roberto F. Escamilla, 655 Sutter Street, San Francisco, California, s in charge of the local arrangements for the meeting.

All Scientific Sessions will be held in the Sir Francis Drake Hotel. The ooms in which each session will be held will be announced in the program nd on the hotel bulletin board. The annual dinner is scheduled for Friday, June 18, at 7:30 P.M., preceded by cocktails at 6:30 P.M.

Hotel reservations may be made through the San Francisco Convention nd Visitors' Bureau, Room 300, Civic Auditorium, San Francisco, California. They should be accompanied by the official application form which has appeared weekly in the J.A.M.A. since December 1953.

Arrangements have been made for members of this society who do not belong to the AMA to be assigned rooms during the days of the meeting of the Endocrine Society. Applications from nonmembers of the AMA should specify dates of arrival and departure and be accompanied by a deposit of $10.00 per room.

Confirmations will not be sent until after February 1, 1954, but *applications should be made promptly* in order to insure the reservation.

PROGRAM

THURSDAY, JUNE 17, 1954

8:30 A.M. **Registration** (*French Parlour, Sir Francis Drake Hotel*)

I. 9:30 A.M. **GONADS, GONADAL HORMONES AND GONADOTROPINS** (*Empire Room*)

Chairmen: Warren O. Nelson and Frederick L. Hisaw

1. Action of Pregnanediols and Related Steroids on Progesterone in Decidual Development. *F. L. Hisaw, J. T. Velardo* (by invitation) and *H. K. Ziel* (by invitation).

2. Control of Menopausal Vasomotor Symptoms with Pituitary Luteinizing Factor and Chorionic Gonadotropin. *A. A. Hellbaum, J. B. Eskridge III* (by invitation) and *R. W. Payne.*

3. Effects of Estradiol on the Metabolism of Human Placenta. *C. A. Ville* (introduced by P. L. Munson).

4. The Metabolism of the Natural Estrogens in Men with Laennec's Cirrhosis of the Liver. *B. F. Stimmel.*

5. Amenorrhea with Normal Follicle-Stimulating Hormone (FSH); Follow-up Report on 60 Cases. *P. H. Benedict* and *F. Albright.*

6. Suppression of Ovulation in the Rat by Morphine. *C. A. Barraclough* (by invitation) and *C. H. Sawyer.*

7. A Sensitive Antihormone Assay Method Employing Chorionic Gonadotropin as the "Test Hormone." *W. O. Maddock, R. B. Leach* (by invitation), I. Tokuyama (by invitation) and *C. A. Paulsen.*

8. Hyperadrenocorticism and the Stein-Leventhal Syndrome. *N. Abu-Haydar* (by invitation), *J. C. Laidlaw* (by invitation), *B. Nusimovich* (by invitation) and *S. H. Sturgis* (by invitation). (Introduced by G. W. Thorn.)

9. Chromatographic Patterns of the 17-Ketosteroid Excretion in Men with Reduced Testicular Activity. *S. Pearson* (by invitation), *T. H. McGavack,* and *H. Haar* (by invitation).

10. The Role of Various Tissues in Metabolism of Androgens. *C. D. Kochakian, D. I. Hamm* (by invitation), *D. M. Nall* (by invitation) and *G. H. Stidworthy* (by invitation).

11. The Kidney β-Glucuronidase Response to Androgens. *W. H. Fishman* (introduced by F. Homburger).

12. Sexual Differences in Nuclei of Human Skin. *E. Marberger* (by invitation) and *W. O. Nelson.*

II. 2:00 P.M. THYROID, THYROID HORMONES AND THYROTROPIN (*Empire Room*)

Chairmen: Paul Starr and Sidney C. Werner

13. Further Evidence that Graves' Disease is Hyperthyroidism and not Hyperpituitarism: Effects of Triiodothyronine and Sodium Iodide. *S. C. Werner, M. Spooner* (by invitation) and *H. Hamilton* (by invitation).

14. The Effect of Iodide on the Rate of Release of Hormone from Toxic Thyroid Glands. *R. E. Goldsmith* (introduced by *V. M. Esselborn*).

15. The Metabolic Effects of Thyrotrophic Hormone. *J. E. Rall, J. Robbins* (by invitation), *C. D. West, O. H. Pearson* and *R. W. Rawson.*

16. An Inquiry into the Levels of Integration of Thyroid Function in Goitrogen Treated Rats. *N. S. Halmi.*

17. Urinary Excretion of Thyrotropic Hormone by Normal Humans and by Subjects with Altered Thyroid Function. *J. P. Kriss* and *F. S. Greenspan.*

18. A Rapid Simple Clinical Radioactive Method for Evaluating Thyroid Function. *W. A. Reilly* (by invitation) and *K. G. Scott* (by invitation). (Introduced by P. H. Forsham.)

19. The Metabolism of Inorganic Iodide in Thyroid Slices with Delineation of a New Iodide Compartment. *J. L. Bakke* and *N. Lawrence* (by invitation).

20. Studies of the Fractional Rate of Release of Hormonal Iodine from the Thyroid Gland. *D. H. Solomon* (introduced by *M. S. Levy*).

21. *In vivo* Interconversion of Thyroxine and Triiodothyronine. *J. R. Hogness, P. VanArsdel, Jr.* (by invitation) and *R. H. Williams.*

22. Studies on the Thyroxine-Protein Complex of Serum. *J. Robbins* (by invitation) and *J. E. Rall.*

23. The Diphasic Character of Thyroidal I^{131} Clearance. *J. H. Nodine* (by invitation), *W. H. Perloff, D. de Albuquerque* (by invitation), *L. Perczek* (by invitation), and *B. Channick* (by invitation).

24. Depression of the Thyroid Iodine Uptake by Hypothalamic Lesions. *W. F. Ganong* (by invitation), *D. S. Fredrickson* (by invitation) and *D. M. Hume* (by invitation). (Introduced by E. Knobil.)

FRIDAY, JUNE 18, 1954

8:30 A.M. Registration (*French Parlour*)

III. 9:00 A.M. ADRENALS, ADRENAL HORMONES AND ADRENOCORTICOTROPINS (*Empire Room*)

Chairmen: Dalton Jenkins and Frank H. Tyler

25. The Diurnal Variation of 17-Hydroxycorticosteroid Levels in Plasma. *F. H. Tyler, C. Migeon* (by invitation), *A. A. Florentin* (by invitation) and *L. T. Samuels.*

26. Correlation of Diurnal Variations in Eosinophils and 17-Hydroxycorticosteroids in Plasma and Urine. *R. P. Doe* (by invitation), *E. B. Flink* and *M. G. Flint* (by invitation).

27. The Functional Diagnosis of Different States of Adrenal Cortical Hyperactivity. *R. Burnett* (by invitation), *M. Rukes* (by invitation), *F. Kolb* and *P. H. Forsham.*

28. An Evaluation of Diagnostic Criteria in Cushing's Syndrome. *A. C. Carter, E. Shorr, T. N. Roberts* (by invitation), *L. S. Sonkin* (by invitation), *R. J. Havel* (by invitation) and *E. T. Livingstone* (by invitation).

29. The Metabolic Pattern Following Alleviation of Cushing's Syndrome. *P. H. Henneman, A. P. Forbes, J. W. Raker* (by invitation) and *F. Albright.*

30. Attempt at Classification of Types of Hirsutism in Women by Chromatographic Analysis of the 17-Ketosteroids. *R. Van de Wiele* (by invitation), *J. W. Jailer, J. J. Gold* (by invitation) and *S. Lieberman* (by invitation).

31. Steroid Isolation Studies in Female Hirsutism: Evidence for Adrenocortical Dysfunction. *A. Kappas* (by invitation), *O. H. Pearson, C. D. West* and *T. F. Gallagher.*

32. Partial Characterization of C-21 Steroids in the Urine of Patients with the Adrenogenital Syndrome. *A. M. Bongiovanni.*

33. Measurement of Dehydroisoandrosterone and Androsterone-Etiocholanolone in Peripheral Plasma of Man. *C. J. Migeon* (introduced by *L. T. Samuels*).

34. The Status of Patients Treated Continuously with Corticotropin for from Three and One-Half Years to over Five Years. *W. Q. Wolfson, C. Cohn* (by invitation), *J. Kennedy* (by invitation), *R. Levy* (by invitation) and *L. Lewis* (by invitation).

35. Effects of Administration of Adrenocorticotropic Hormone and Cortisone During Pregnancy upon Mothers, Developing Fetuses and Infants. *R. R. Margulis, C. P. Hodgkinson, P. J. Howard* (by invitation) and *E. I. Gordon* (by invitation).

IV. 1:30 P.M. ANNUAL BUSINESS MEETING OF THE ENDOCRINE SOCIETY (*Empire Room*)

(All members requested to attend promptly).

V. 2:30 P.M. ADRENALS, ADRENAL HORMONES AND ADRENOCORTICOTROPINS (*Empire Room*)

Chairmen: Ralph I. Dorfman and John A. Luetscher, Jr.

36. The Steroid 11-β-Hydroxylase. *M. Hayano* (by invitation) and *R. I. Dorfman.*

37. The Metabolism and Excretion of Intravenously Administered 4-C^{14}-17-Hydroxycorticosterone, *J. E. Plager* (by invitation), *F. H. Tyler, H. H. Hecht* (by invitation) and *L. T. Samuels.*

38. Interrelationships of Liver Glycogen Deposition, Corticosteroids and Ascorbic Acid in Scorbutic Guinea Pigs. *A. S. Mulay* (by invitation), *L. D. Saslaw* (by invitation) and *E. M. Nadel.*

39. A New Method for the Determination of ACTH in Blood. *D. H. Nelson* and *D. M. Hume* (by invitation).

40. Adrenocorticotropic Substances from Human Placenta. *N. S. Assali* (by invitation) and *J. Hamermesz* (by invitation). (Introduced by C. H. Sawyer.)

41. Corticosteroid Secretion Following Hypophysectomy. *M. L. Sweat* and *G. L. Farrell* (by invitation).

42. Chlorohydrocortisone Acetate, A Highly Potent Derivative of Compound F. *A. Goldfien* (by invitation), *P. M. Beigelman* (by invitation), *J. C. Laidlaw* (by invitation) and *G. W. Thorn.*

43. Factors Regulating the Output of Sodium-Retaining Corticoid of Human Urine. *B. J. Axelrad* (by invitation), *B. B. Johnson* (by invitation) and *J. A. Luetscher, Jr.*

44. Studies on Electrocortin. *R. Gaunt, A. S. Gordon, A. A. Renzi* (by invitation), *J. Padawer* (by invitation), *G. J. Fruhman* (by invitation) and *M. Gilman* (by invitation).

45. Excretion of Sodium Retaining Substances. *E. H. Venning, A. Carballeira* (by invitation) and *I. Dyrenfurth* (by invitation).

VI. ANNUAL DINNER

Earl T. Engle, President, presiding

6:30 P.M. Cocktails (*French Parlour*)

Sponsored by the donors of the Awards of the Society: Ayerst, McKenna and Harrison, Ciba, and Schering. Attendance limited to holders of dinner tickets.

7:30 P.M. Dinner (*Empire Room*)

Presentation of the 1954 Award and Fellowship of the Society by Carl Moore, Chairman of the Committee on Awards:
The Ciba Award
The Ayerst, McKenna and Harrison Fellowship

President's Address: Dr. Engle

SATURDAY, JUNE 19, 1954

8:30 A.M. Registration (*French Parlour*)

VII. 9:00 A.M. PARATHYROID, PITUITARY AND GROWTH HORMONES (*Empire Room*)

Chairmen: Roberto F. Escamilla and Arthur Grollman

46. Endocrinologic Studies on the Nephrectomized Dog. *A. Grollman.*

47. Effects of Benemid (Probenecid) in the Treatment of Hypoparathyroidism and Pseudo-Hypoparathyroidism. *F. O. Kolb* and *J. M. Rukes* (by invitation).

48. Phosphate-Losing Osteomalacia, and its Paradoxical Relief by Large Doses of Vitamin D. *R. Fraser* (by invitation) and *B. E. C. Nordin* (by invitation). Introduced by G. S. Gordon.)

49. The Fine Structure of the Anterior Pituitary Gland as Revealed by Electron Microscopy. *M. G. Farquhar* (by invitation) and *J. F. Rinehart* (by invitation). (Introduced by R. F. Escamilla.)

50. Physiological Effects of Hypophysectomy in Man. *C. D. West, M. C. Li* (by invitation), *J. P. Maclean* (by invitation), *J. E. Rall* and *O. H. Pearson.*

51. Growth Hormone and Lymphatic Organs. *L. F. Kumagai* (by invitation), *B. A. Brill* (by invitation) and *T. F. Dougherty.*

52. Growth Hormone in Normal and Hypophysectomized Immature Rhesus Monkeys. *E. Knobil, W. F. Ganong* (by invitation) and *R. O. Greep.*

53. The Inhibition of the Fat Mobilizing Properties of Ethionine by Anabolic Steroids and Growth Hormone. *A. Segaloff* and *E. Farber* (by invitation).

54. Antidiuretic Activity of Normal and Hypophysectomized Rats. *C. W. Lloyd, S. Pierog* (by invitation), *E. H. Loewy* (by invitation), *K. Bradwick* (by invitation) and *R. Sostheim* (by invitation).

55. Hereditary Diabetes Insipidus, with Report of Many Instances in Six Generations of One Family. *E. L. Levinger* (by invitation) and *R. F. Escamilla.*

56. Melanocyte Stimulating Hormone (Intermedin, Melanophore Hormone) in Human Blood and Urine. *K. Shizume* (by invitation), *A. B. Lerner* (by invitation) and *T. B. Fitzpatrick* (by invitation). (Introduced by P. H. Forsham.)

57. Endocrine Control of an Induced Hepatic Enzyme System. *I. I. Geschwind* and *C. H. Li.*

VIII. 2:00 P.M. JOINT SESSION WITH THE AMERICAN DIABETES ASSOCIATION. (*Sir Frances Drake Hotel, Empire Room*)

Chairmen: Randall G. Sprague and Earl T. Engle

58. Hypophysectomy in Patients with Malignant Diabetic Vascular Disease: Metabolic and Clinical Evaluation. *L. W. Kinsell, N. Foreman* (by invitation), *H. E. Balch* (by invitation) and *M. Coelho* (by invitation).

59. Increased Insulin Sensitivity in Rats with Hypothalamic Lesions and its Interpretation. *B. N. Spirtos* (by invitation), *W. R. Ingram* (by invitation), *E. M. Bogdanove* (by invitation) and *N. S. Halmi.*

60. Some Effects of Growth Hormone on the Structure and Function of the Islets of Langerhans. *L. L. Bennett, J. H. R. Sutherland* (by invitation) and *A. A. Batts* (by invitation).

61. Metabolic Adjustments During Stress: The Effect of Intravenous Glucose on Pyruvate During Compound F Infusion. *T. F. Frawley, J. J. Garrett* (by invitation) and *P. J. Rosch* (by invitation).

62. The Ketogenic Activity of Corticotropin in the Rat. *F. L. Engel* and *M. G. Engel* (by invitation).

The American Goiter Association

THE 1954 ANNUAL MEETING

The annual meeting will be held at the Somerset Hotel, Boston, Massachusetts, April 29, 30 and May 1, 1954. It is suggested that you make your hotel reservations by writing Mr. Harry Hoghn, Executive Assistant Manager, The Somerset, 400 Commonwealth Avenue, Boston 15, Massachusetts.

JOHN C. MCCLINTOCK, M.D.,
Corresponding and Recording Secretary

(For program, *see* March issue of the Journal)

BOOKS RECEIVED

American Lecture Series. Charles C Thomas, Publisher, Springfield, Ill.

The Action of Insulin. By NIELS HAUGAARD, PH.D., Asst. Prof. of Physiological Chemistry in Research Medicine, Univ. of Pennsylvania, Philadelphia, Pa., and JULIAN B. MARSH, M.D., Asst. Prof. of Physiological Chemistry, Graduate School of Medicine, Univ. of Pennsylvania. A monograph in Am. Lectures in Endocrinology, edited by WILLARD O. THOMPSON, M.D.; 120 pages; 8 figures; 6 tables; 1953. Price $3.75.

Copying and Duplicating Medical Subjects and Radiographs. By H. LOU GIBSON, Technical Editor, Eastman Kodak Company, Rochester, N. Y. A monograph in Am. Lectures in Medical Photography, edited by RALPH P. CREER; 88 pages; 42 illust. (1 in color); 1953. Price $5.00.

Hyperparathyroidism. By B. MARDEN BLACK, M.D., Assoc. Prof. of Surgery, Mayo Foundation Graduate School, University of Minnesota, and Mayo Clinic, Rochester, Minn. A monograph in Am. Lectures in Endocrinology, edited by WILLARD O. THOMPSON, M.D.; 127 pages; 24 illust.; 1953. Price $3.75.

Hypertensive Disorders of Pregnancy. By ERNEST W. PAGE, M. D., Assoc. Prof. Obstetrics and Gynecology, Univ. of California School of Medicine, San Francisco, Calif. A monograph in Am. Lectures in Circulation, edited by IRVINE H. PAGE, M.D. and A. C. CORCORAN, M.D.; 135 pages; 2 illust.; 1953. Price $3.75.

Practical Management of Diabetes. By EDWARD TOLSTOI, M.D., Assoc. Prof. of Clin. Med., Cornell Univ. Medical College; Att. Physician and Chief, Diabetic Hospital, The New York Hospital, New York, N.Y. A monograph in Am. Lectures in Metabolism, edited by P. GYORGY,

M.D., L. LEITER, M.D. and S. O. WAIFE, M.D.; 112 pages; 1953. Price $3.25.

Size and Growth of Tissue Cells. By JOSEPH G. HOFFMAN, PH.D., Director of Cancer Research, Roswell Park Memorial Institute, Res. Prof. of Biophysics, School of Medicine, Univ. of Buffalo, Buffalo, N.Y. A monograph in Am. Lectures in Medical Physics, edited by OTTO GLASSER, PH.D.; 128 pages; 13 illust.; 5 tables; 1953. Price $4.00.

Treatment of Toxic Goiter With Radioactive Iodine. By LINDON SEED, M.D., Director, Isotope Laboratory, Grant Hospital, Prof. of Surgery, College of Medicine, Univ. of Illinois, and THEODORE FIELDS, M.S., Asst. Director, Radioisotope Laboratory, Hines Hospital, Instructor in Radiology, Northwestern Univ. Medical School, Chicago, Ill. A monograph in Am. Lectures in Endocrinology, edited by WILLARD O. THOMPSON, M.D.; 128 pages; 17 illust.; 6 tables; 1953. Price $3.75.

Ciba Foundation Colloquia on Endocrinology. Little Brown & Company, Boston, Mass.

Vol. VI. Hormonal Factors in Carbohydrate Metabolism. Chairman, C. H. BEST, M.D., Dept. of Physiology, Univ. of Toronto, Canada. Edited by G. E. W. WOLSTENHOLME, assisted by J. S. FREEMAN; 364 pages; 94 illust.; 1953. Price $6.75.

Vol. VII. Synthesis and Metabolism of Adrenocortical Steroids. Consulting Editor, W. KLYNE, PH.D.; Editors, G. E. W. WOLSTENHOLME and MARGARET P. CAMERON; 315 pages; 29 illust.; 1953. Price $6.75.

Clinical Endocrinology. By ALLAN WILLIAM SPENCE, M.A., M.D., F.R.C.P., Physician, St. Bartholomew's Hospital, London, England; 708 pages; 59 figures; 2 color plates; extensive bibliography; 1953. Paul B. Hoeber, Inc., New York. Price, $12.50.

Determination of Adrenocortical Steroids and Their Metabolites. Memoirs of the Society for Endocrinology, No. 2. Proceedings of a conference held by the Society for Endocrinology at the Medical Society of London, May 21, 1953; edited by P. ECKSTEIN and S. ZUCKERMAN; 96 pages; figures and tables; 1953. Dennis Dobson, Ltd., 12 Park Place, St. James's, London SW1. Price 12s 6d net.

(Le) diabète alloxanique: contribution a l'étude de la pathogénie du syndrome. By P. DE MOOR, Agrégé de l'Enseignement Supérieur, Anvers (Belgique); 206 pages; figures and tables; English summary; extensive bibliography; 1953. Masson et Cie, Paris.

Enzymatic Concept of Anaphylaxis and Allergy and the Role of Eosinophils in Anaphylactic Reactions Related to Hormonal Alterations. By Z. Z. GODLOWSKI, M.D., PH.D., Research Fellow of the Carnegie Trust, Edinburgh; Acting Prof. of Physiopathology and Lecturer in Clin. Medicine at the late Polish School of Medicine, Edinburgh University, Scotland., with a foreword by A. Murray Drennan, M.D., Prof. of Pathology, Univ. of Edinburgh; 128 pages; 7 tables; 17 illust. (some in color), 1953. E. & S. Livingstone, Ltd., Edinburgh and London; The Williams & Wilkins Co., Baltimore, Md. Price $3.50.

Fundamentals of Biochemistry in Clinical Medicine. By NIELS C. KLENDSHOJ, M.D., Asst. Prof. of Pathology, Univ. of Buffalo School of Medicine and Director of

the Dept. of Biochemistry, Buffalo General Hospital, Buffalo, N. Y.; 288 pages; 9 illust.; 1953. Charles C Thomas, Publisher, Springfield, Ill. Price $7.75.

Metabolism of Steroid Hormones. By RALPH I. DORFMAN, PH.D., Res. Prof. Biochemistry, Boston Univ., Boston, Mass., and FRANK UNGAR, PH.D.—both of The Worcester Foundation for Experimental Biology, Shrewsbury, Mass.; 173 pages; illustrated; 1953. Burgess Publishing Co., Minneapolis, Minn. Price $4.00.

Pregnancy Wastage. Proceedings of a Conference sponsored by the Committee on Human Reproduction, National Research Council in behalf of the Nat. Committee on Maternal Health, Inc. Edited by EARL T. ENGLE, Dept. of Obstetrics & Gynecology, Columbia Univ. College of Physicians & Surgeons, New York, N. Y.; 272 pages; figures and tables; 1953. Charles C Thomas, Publisher, Springfield, Ill. Price $8.50.

A Source-Book of Medical Terms. By EDMUND C. JAEGER, D.SC., formerly Head of Dept. of Zoology, Riverside College, Riverside, Calif.; with a foreword by IRVINE H. PAGE, M.D.; 176 pages; illustrated by LLOYD MASON SMITH and the author; 1953. Charles C Thomas, Publisher, Springfield, Ill. Price $5.50.

(La) sterilità nel maschio: fisiopatologia degli organi della riproduzione diagnostica e terapia dell' infertilità maschile. GUGLIELMO LONGO, Assistant della Scuola di Ostetricia di Salerno; 540 p.; 83 illust.; 1953. Diffusion Scientifique Internationale, Salerno, Italy. Prezzo: L. 4000.

Thyreotoxikosen und antithyreoidale Substanzen. VON HANS WILHELM BANSI, Apl. Prof. an der Univ. Hamburg, Chefarzt der I. Med. Abtlg. des allg. Krankenhauses St. Georg, Hamburg, Germany; 100 Seiten, 4 Abbild.; 1951. Georg Thieme Verlag, Stuttgart, Germany, Preis: Kartoniert DM 11.40.

Transactions of Josiah Macy, Jr. Foundation Conferences, New York, N. Y.
Adrenal Cortex. Fourth Conference, Nov., 1952. Edited by ELAINE P. RALLI; 165 pages; 22 figures; 9 tables; 1953. Price $4.00.